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New Pest Response Guidelines

Candidatus Liberibacter africanus *Ca.* L. asiaticus, *Ca.* L. americanus

Huanglongbing
Citrus Greening Disease

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Version 1.1 May 2006

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CONTENTS

SECTION	PAGE
ACKNOWLEDGEMENTS	ii
CONTENTS	iii
LIST OF TABLES AND FIGURES	iv
1. INTRODUCTION	
o Purpose	1.1
o Disclaimer	1.1
o The Citrus Health Response Plan	1.1
o HLB Infection Prevention	1.2
o Program Safety	1.2
o Support for Program Decision Making	1.2
2. PEST INFORMATION	
o Nomenclature	2.1
o Background Information	2.1
o Historical Information	2.3
o Biology of *Candidatus* Liberibacter	2.3
o Biology of Psyllid Vectors	2.4
o Economic Impact	2.6
o Plant Hosts	2.6
o Geographic Distribution	2.7
3. SURVEY PROCEDURES	
o Sanitation Precautions for Inspectors	3.1
o General Detection Survey	3.1
o Symptomology for Inspections During Detection Surveys	3.2
o Traceback/traceforward Investigations	3.3
o Delimiting Survey after Initial US Detection	3.4
o Rapid Delimiting Survey	3.5
o Stratified Surveys Over Large Growing Areas	3.7
o Vector Surveys for Predicting Future Outbreaks	3.7
o Guidelines for Residential Surveys	3.7
o Guidelines for Commercial Grove Survey	3.8
o Guidelines for Nursery Survey	3.10
o Other Hosts Surveys	3.11
o Monitoring Surveys	3.11
4. DIAGNOSTICS AND IDENTIFICATION	
o Importance	4.1
o Authorities	4.1
o Centralized National Survey Screening Laboratory	4.2
o Classification of Plant Sample Symptoms	4.3

- Sample Storage and Forwarding 4.3
- Psyllid Samples 4.4
- Sample Packaging and Documentation 4.4
- Sample Labeling, Numbering, and Record Keeping 4.4
- Centralized National Screening Laboratory Results 4.5
- Web-based Reporting Tool 4.5
- State or other Diagnostic Screening Laboratory Results 4.5
- Completing the PPQ form 391 Determination Section 4.7
- Saturday Delivery to the NPGLB 4.7
- Notification of State Officials of Sample Submissions and Results 4.7
- Sample Processing Time 4.8

5. REGULATORY PROCEDURES
   - Instructions to Officers 5.1
   - Quarantine Actions and Authorities 5.1
   - Overview of Regulatory Program for Citrus Greening after a US Detection 5.2
   - Record Keeping 5.2
   - Issuing an Emergency Action Notification 5.2
   - Regulated Articles 5.4
   - Huanglongbing Hosts 5.4
   - Citrus Psyllid Hosts 5.4
   - Shipment of Insect Vectors Hosts 5.5
   - Regulatory Treatments for Psyllid Hosts Nurserystock 5.5
   - Quarantine Area 5.6
   - Grower Requirements Under Quarantine 5.6
   - Establishing a Federal Quarantine 5.7
   - Removing Areas from Quarantine 5.7
   - Regulatory Records 5.7
   - Use of Chemicals 5.7

6. CONTROL
   - Overview 6.1
   - The Citrus Health Response Plan 6.1
   - Control Decisions and Oversight 6.1
   - Control of the Huanglongbing Disease 6.1
   - Control of the Psyllid Vectors 6.3
   - Control Procedures for Positive Nursery Detections 6.5
   - Tree Removal and Disposal 6.5
   - Control of Hosts of Insect Vectors 6.5
   - Biological Control of Psyllid Vectors 6.6
   - Long-term HLB Management 6.7
   - Control Records 6.7
   - Environmental Monitoring 6.7

7. ENVIRONMENTAL CONCERNS
- Overview 7.1
- Disclaimer 7.1
- National Environmental Policy Act 7.1
- Categorical Exclusion 7.1
- Environmental Impact Statements 7.2
- Environmental Assessments 7.2
- Environmental Monitoring 7.3
- Biological Assessment 7.3

8. DEFINITIONS 8.1

9. APPENDIX 1 Psyllid Vectors and HLB Symptoms on Hosts 9.1

10. APPENDIX 2 Insect Vector Identification 10.1

11. APPENDIX 3 Disinfection Procedures 11.1

12. APPENDIX 4 Forms
PPQ form 391, Specimen for Determination 12.1
PPQ form 523, Emergency Action Notification 12.2

13. APPENDIX 5 DNA Extraction and PCR Detection in Citrus 13.1

14. APPENDIX 6 Registered Insecticides for Control of Citrus Psyllids 14.1

15. APPENDIX 7 References 15.1

TABLES AND FIGURES	PAGE
Figure 1. Sampling points along concentric annuli transects at 5 mile increments away from a known positive host tree.	3.6
Figure 2 Sampling points along an arc transect showing where searching begins to find the nearest host tree for survey.	3.6
Figure 3. If the disease is not found in the transect sampling point, travel back toward center sampling in one mile increments.	3.6
Figure 4. If disease is found to extend beyond an annulus in one or two directions, use a partial annulus combined with information from other transects do define the boundaries of the infestation.	3.6
Figure 5. Diagram showing a defined infected area after a delimiting survey, buffer of exposed hosts removed, and area outside that area where other stratified surveys are conducted.	3.7
Table 1. Hosts of *Candidatus* Liberbacter , with some indications of susceptibility	5.4
Table 2. Hosts of *Diaphoria citri* and not *Candidatus* Liberbacter	5.6

INTRODUCTION

Purpose

This New Pest Response Guidelines presents available information for designing a site specific action plan implementing detection, diagnosis, containment and control or eradication of Huanglongbing disease (HLB, or citrus greening). Specific emergency program activity should be based on information available at that time.

The document provides background information on the disease and its hosts, the causal pathogens, and the vectors that carry the pathogens. The control approach is an amalgam of methods employed for HLB control in other countries and methods used to control other vector-transmitted pathogens of citrus in the United States. It is intended to provide a starting point for a control/eradication program, with modifications being made as the program develops.

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) agency developed these guidelines through discussion, consultation, or agreement with other APHIS staff, the Agricultural Research Service (ARS), university advisors, States, and industry. It is to be used in conjunction with other agency regulations, guidelines, and manuals when conducting program activities. The information contained in these guidelines is based on the best scientific information available at the time of writing in consultation with States and industry. The guidelines will be updated as new information becomes available. Specific emergency program actions should be based on the best information available at the time of the incident.

Disclaimers

Document Comprehensiveness: This document is not intended to be complete and exhaustive, but provides a foundation, based upon the literature available, to assist further work. Some key publications were not available at the time of writing, and not all specialists and members of the research community were consulted.

Commercial Suppliers or Products: Any references to commercial suppliers or products should not be construed as an endorsement of the company or product by the USDA.

The Citrus Health Response Plan

The Citrus Health Response Plan in development in 2006 in Florida will have recommendations and a regulatory component that includes long-term management practices for HLB while maintaining production and commerce. The procedures developed as a part of that process will provide further guidelines on HLB disease management.
To see the draft currently available for comment, go to:
http://www.aphis.usda.gov/ppq/pdmp/citrushealth/

HLB Infection Prevention

Federal and state regulatory officers must conduct inspections and apply prescribed measures to ensure that the disease or pathogen does not spread within or between properties. Since inspectors could inadvertently spread HLB or other pathogens through the inspection and sampling process, federal and state regulatory officers conducting inspections should follow the sanitation guidelines in the beginning of the Survey section to prevent spreading contaminated plant material or tools to other facilities before entering and upon leaving each property.

Program Safety

The safety of the public as well as the program personnel is a priority consideration in preprogram planning and training, and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decision-Making

The USDA/APHIS/PPQ Center for Plant Health, Science and Technology (CPHST) provides technical support, in consultation with other scientists, to emergency pest response program directors concerning risk assessments, survey methods, control strategies, and other aspects of pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policy for pest response programs.

PEST INFORMATION

Nomenclature

Domain: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rhizobiales
Family: Rhizobiaceae

Scientific Names: *Candidatus* Liberibacter africanus Garnier
Candidatus Liberibacter asiaticus Garnier
Candidatus Liberibacter americanus *sp. nov.* (Texiera, et al., 2005)

Synonyms: *Candidatus* Liberobacter africanus Garnier
Candidatus Liberobacter asiaticus Garnier

Approved common name (IOCV, 1996):

Huanglongbing (yellow shoot or, literally, "yellow dragon" [China])

Additional common names:

HLB, likubin or decline (Taiwan), leaf mottling (Philippines), citrus dieback (India), vein phloem degeneration (Indonesia), yellow branch or blotchy-mottle (South Africa)

Nomenclature

The term *Candidatus* is used for bacteria species that have not yet been successfully cultured, so a taxonomic description cannot yet be completed. If *Candidatus* is used, the actual genus and species are not italicized. (Halbert and Manjunath 2004). Garnier et al. (2000) changed the generic name from Liberobacter to Liberibacter, following the International Code of Nomenclature of Bacteria, which states that since "bacter" is of masculine gender and "Liber" is of Latin origin, the connecting vowel should be an "i."

Background Information

Huanglongbing, (HLB, or citrus greening) disease is a serious bacterial disease of citrus that greatly reduces production, destroys the economic value of the fruit, and can kill trees. The HLB organisms, *Candidatus* Liberibacter asiaticum Garnier and *Candidatus* Liberibacter africanum Garnier are listed as select agents under the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). *Candidatus* Liberibacter

americanum was recently described from Sao Paulo, Brazil (Texiera et al. 2005, Coletta-Filho et.al. 2005) and is not listed as a select agent.

HLB is a bacterial disease vectored by two species of citrus psyllids: *Diaphorina citri* Kuwayama and *Trioza erytreae* (del Guercio), respectively (Aubert 1987). The bacteria are phloem–limited and cause yellow shoots, blotchy mottling and chlorosis, reduced foliage and tip dieback of citrus (Bové and Garnier 2002). Citrus fruits are lopsided, small, remain green with seeds aborted (Bové and Garnier 2002) and have a sour taste. The disease is graft- and vector-transmissible (Catling 1970; Catling 1973; Ghosh et al. 1977; Garnier and Bové 1983; Garnier et al. 1984b; Garnier et al. 1984a; da Graça 1991; Swai et al. 1992; Kohno et al. 2001).

The name "Huanglongbing" was adopted as the official name of the disease by the International Organization of Citrus Virologists (IOCV) (at the 12th Congress of IOCV, Fuzhou, China, 1995) (Garnier et al., 1984a; Bové and Garnier 2002).

The host range of the bacteria under natural conditions appears to be restricted to rutaceous plants, although dodder, periwinkle (Tirtawidjaja, 1981), and tobacco (Garnier and Bové 1993) have been infected under experimental conditions. It severely affects sweet orange, mandarin and tangelo trees, but susceptibility among other *Citrus* species varies (Garnier et al. 1984a; Bové and Garnier 2002). Mexican lime (*Citrus aurantifolia*) is less susceptible than sweet orange and mandarin even though it is a preferred host of the vector *Diaphorina citri* (Garnier et al. 1984a; Bové and Garnier 2002). Several wild and ornamental rutaceous species are hosts of the psyllid vectors, such as the orange jasmine *Murraya paniculata* and curry leaf tree, *Murraya keonegii*, however the literature is inconclusive as to *Murraya* species status as hosts for the HLB pathogens (Halbert and Manjunath 2004).

The bacteria can be transmitted in orchards or nurseries by grafting and experimentally by several species of dodder (*Cuscuta* spp.) (Halbert and Majunath, 2004). Natural transmission of *Ca.* L. africanum is facilitated by both the African citrus psyllid *Trioza erytreae*. *Ca.* L. asiaticus and *Ca.* L. americanus are vectored by the Asian citrus psyllid *Diaphorina citri* (Teixeira et. Al. 2005). However, each psyllid species has been demonstrated to either the African of Asian form of the bacteria experimentally.

The bacteria reproduce in the hemolymph and salivary glands of the insects after they feed on infected plants (Catling 1970; Garnier et al. 1984a; Aubert 1987; Rae et al. 1997; Subandiyah et al. 2000; Bové and Garnier 2002). Once the citrus psyllids acquire the bacteria, they

transmit it to new hosts for the remainder of their life cycle in a persistent, propagative manner (Garnier et al., 1984a; da Graça 1991; Bové and Garnier, 2002). It is not known if the bacteria are transmitted trans-ovarially to subsequent generations, but the nymphs and adults can acquire the bacteria in less than one day (Aubert, 1987).

Incubation periods in the vector range from as little as one day (African form) to as long as 12 days (Asian form). Adults are then capable of transmitting the bacteria for the remainder of their lives (Aubert 1987). The 2th through 5th instar nymphs can also acquire and, under experimental conditions, the 4th and 5th instars can transmit the bacteria. Only adult psyllids are known to transmit the bacteria under natural conditions. The form described in Brazil (the American form) appears to be transmitted by the Asian citrus psyllid, which is common in Brazil, or by grafting (Coletta-Filho et al. 2005).

The three forms of greening produce similar symptoms in citrus, although the Asian form usually produces a more severe disease reaction than the African form. Bacterial strain differences affecting symptom severity have been noted in both the African and Asian forms. The African form of the pathogen is heat sensitive, with symptoms produced under relatively cool conditions (20-24^{o} C optimum) (Garnier and Bové, 1993). Extended periods of high temperatures suppress symptom development. The Asian form is heat tolerant, producing symptoms under cool to relatively warm conditions (up to 32^{o} C) (Bové et al. 1974). Research is underway to more fully characterize the Brazilian form.

Historical Information

A review by da Graça and Korsten (2004) provides useful information on HLB's history. Farmers in southern China noted a citrus disease, which they called "yellow dragon" (huanglongbing), in the late 1800's. In the early 1920's, similar diseases were reported in the Philippines (mottle leaf), India (dieback), and Taiwan (likubin). A citrus disease, called yellow branch or greening, was noted in South Africa in the late 1920's, and a malady known as citrus phloem degeneration, was reported from Indonesia in the 1940's. The disease has subsequently been found in numerous other citrus producing countries in Africa and Asia.

It was not until the 1960's that the link among these various diseases was established. The disease was initially believed to be caused by drainage problems, while later reports suggested that a mineral deficiency was the cause. Likubin, in Taiwan, was reported to be associated with a nematode problem. Successful graft transmission of the disorder in the 1940's and insect transmission in the 1960's led to speculation that the causal agent was a virus. In 1970, mycoplasma-like

organisms (or phytoplasmas) were putatively observed in the sieve tubes of greening-infected orange leaves. More recent molecular studies have established that the causal organisms are actually true bacteria (da Graça, 1991).

The disease has subsequently been found in numerous other citrus producing countries in Africa and Asia. The first report of the disease in the Western Hemisphere came from Brazil in 2004; confirmation of the disease in Florida was announced in a press release on September 2, 2005 (USDA 2005).

Biology of *Candidatus* Liberibacter

The bacteria have not yet been successfully cultured outside of the phloem of citrus plants or the psyllid vectors (Garnier et al., 1984a; da Graca, 1991; Bové and Garnier, 2002). The African strain of HLB, present in South Africa, Kenya, Ethiopia, Madagascar and Yemen, is heat sensitive and unable to cause symptoms at temperatures above 25-30°C, whereas, the Asian strain, *Ca*. L. asiaticum, which occurs throughout much of Asia, India, and Indonesia, is heat-tolerant and able to cause symptoms at temperatures above 30°C (Garnier et al., 1984a; da Graça, 1991; Bové and Garnier, 2002).

The liberibacters inhabit the nutrient-rich phloem. Other similar organisms cause more than twenty diseases of plants, including papaya bunchy top, watermelon yellow vine, and strawberry marginal necrosis (Kiritani and Su, 1999; Bové and Garnier, 2002). None of these organisms have been cultured (Bové and Garnier, 2002).

When the 16s ribosomal DNA of *Ca.* L. africanum was amplified, the DNA was most similar to the bacteria of the α subdivision of *Proteobacteria*, which include plant and human pathogens such as *Agrobacterium tumefaciens, Bradyrhizobium* spp., and *Brucella abortus* (Jagoueix et al., 1994; Bové and Garnier, 2002).

Biology of Psyllid Vectors

The African citrus psyllid *Trioza erytreae* and the Asian citrus psyllid *Diaphorina citri* are the only known insect vectors of the greening pathogens. Each of these vectors can transmit both *Ca*. L. africanus and *Ca.* L. asiaticus, experimentally, however in nature each psyllid has only been found associated with their respective bacteria as previously described.

The Asian citrus psyllid, *Diaphorina citri*, is now widespread throughout the citrus growing areas of Florida (Halbert et al. 1998, Tsai et al. 2002) and the lower Rio Grande Valley of Texas (French et al. 2001). The host range of *Diaphorina citri* is restricted to citrus and closely related Rutaceae (Aubert 1987, Halbert and Manjunath 2004). The preferred host is *Murraya paniculata*, an ornamental rutaceous plant called orange

jasmine (also known as orange jessamine and commonly in the Florida nursery trade as "Lakeview", a cultivar) found throughout the citrus belt in its native range (Kohno et al. 2001) and often planted in the Southeastern United States as an ornamental hedge plant. The Asian citrus psyllid recently spread to all citrus growing areas in Florida with the host plant (Halbert and Majunath 2004). However, orange jasmine is not currently considered a host for the liberibacters (Hung et al., 2000).

Adult citrus psyllids are small (3 to 4 mm) with mottled brown wings. Adults are active, jumping insects. Eggs are bright yellow and deposited on newly emerging citrus tissue. Nymphs are green or dull orange, and feed on leaves and stems where they are difficult to see. Asian citrus psyllids are most likely to be found on new shoots, and population increase occurs during periods of active plant growth (Aubert 1987).

D. citri has a light brown head, while *Trioza erytreae* adults have a black head. When disturbed, the adult psyllids move or jump quickly, occasionally flying short distances. Nymphs of *Diaphorina. citri* are light yellow to dark brown, with large, well-developed wing pads. Nymphs of *Trioza erytreae* vary in color from yellow to olive-green to dark gray, with marginal fringes of white, waxy filaments and small wing pads. Additional information on psyllid identification can be found in Appendix 2.

High populations of *Diaphorina citri* stunt and twist young shoots, causing a rosette appearance. Leaves are badly curled, but do not contain the pit galls typical of *Trioza erytreae*. High populations of *Trioza erytreae* severely distort leaves, which are stunted and contain typical pit galls.

Psyllid populations increase in late winter and spring when the citrus trees are flushing (new foliage growth) and adults may fly for short distances. Cool, moist conditions favor increased populations of *Trioza erytreae*, while *Diaphorina citri* prefers a warmer, drier environment. High psyllid populations are often found in citrus nurseries, since the young trees are maintained in a state of almost constant growth. Active growth on alternate plant hosts support psyllid populations when citrus flush is not available.

The interaction between the vector and the pathogen is poorly understood. Acquisition times of between 30 minutes and 24 hours have been reported (Aubert 1987). The pathogen also multiplies in the vector. Adults and fourth and fifth instar Asian citrus psyllid nymphs can transmit *Ca*. L. asiaticum after 8-12 days, with a shorter latent period of 1 day reported for African psyllids. Following a 1-21 day incubation period (average 7-12 days), the psyllids are able to transmit the bacteria

for the rest of their lives (average lifespan of 60 days). A 2nd or 3rd instar could acquire the pathogen and become increasingly infective in the 4th or 5th instar. (Hung et al. 2004). Nymphs, however, would remain on the HLB-infected host material from which it acquired the *Ca*. L. asiaticus pathogen and pose little risk of spreading the disease to new material until reaching the adult stage.

Recent experiments by Hung et al. 2004 show the pathogen not to be transmitted transovarially (from adult to egg).

Nymphs reportedly do not transmit the pathogen in the field, although they are able to acquire it when feeding on infected plants and later transmit it as adults. Late-instar nymphs are, however, able to transmit the bacteria when moved from infected to healthy plants under experimental conditions. Most adult insect movement is within the host plant or to nearby plants, but the psyllids are capable of flying considerable distances (1.5 miles has been documented) in search of suitable hosts.

NOTE: not all plants the psyllids feed on are hosts of the bacteria. For a complete list of psyllid hosts, see Halbert and Manjunath 2004.

Economic Impact

By the early 1990's, HLB had become widespread throughout the citrus growing regions of Asia and the southern and eastern parts of Africa, and resulted in the estimated loss of over 60 million citrus trees by the early 1990's. The following examples serve to illustrate the seriousness of both the Asian and African forms of this disease.

Four million citrus trees were eradicated on the island of Bali from 1986 to 1988. These trees were replaced with mandarins in 1991. By 1993, 40% of the replacements were infected with greening, increasing to a 90% infection rate by 1996. The area planted to citrus in the Philippines was reduced by over 60% between 1961 and 1970 due to greening, with a loss of over one million trees recorded in one province in 1971. Many trees in Thailand die or go out of production within 5-6 years after planting.

An estimated 4 million of the 11 million citrus trees planted in South Africa were infected with greening by the mid 1970's. By this time, three major production areas, representing 20% of the citrus industry, had been eliminated due to the disease.

Plant Hosts

Rutaceous plants are the natural hosts of Liberibacter species, with all species and cultivars of citrus susceptible to infection (Garnier et al. 1984; Bove and Garnier 2002). Sweet oranges (*Citrus sinensis*), mandarins (*C. reticulata*), and mandarin hybrids are the most severely

affected. Grapefruit (*C. x paradisi*), sour oranges (*C. aurantium*), and lemons (*C. limon*) are moderately affected. Mexican (Key) lime (*C. aurantifolia*), pummelo (*C. maxima*) and trifoliate orange (*Poncirus trifoliata* Raf.), including its hybrids, are the most tolerant. Kumquat (*Fortunella spp.*) is also a host.

A number of other rutaceous plants have been observed to be hosts of both the bacteria and the citrus psyllid, either experimentally or naturally: *Severinia buxifolia, Balsamocitrus dawei, C. grandis, C. hystrix, C. jambhiri, Citrus* x *nobilis, Clausena indica, Cl. lansium, Microcitrus australisica, Triphasia trifolia, Atalantia missionis, Severinia buxifolia, Limonia acidissima* (= *Feronia limonia*), and *Swinglea glutinosa* (Hung et al. 2000; Hung et al. 2001; Halbert and Manjunath 2004).

Several wild and ornamental rutaceous species are hosts of the psyllid vectors, such as orange jasmine (*Murraya paniculata*), curry leaf plant (*Murraya keonegii*), jackfruit, *Artocarpus heterophyllus,* and cape chestnut, *Calodendrum capense* Thunb. It is not known conclusively if the *Murraya* spp. hosts for the vector are also host for the pathogen, but evidence so far suggests it is not. Specimens of *Calodendrum capense* were found that were infected with a distinct subspecies of *L. africanus*.

Some of these ornamentals may be used in warm climate areas of the United States. Orange jasmine is a preferred host of the Asian citrus psyllid, and has likely allowed the spread of this insect throughout the citrus growing areas of Florida and Texas. The plant *Toddalia lanceolata* (=*Vepris undulata*), considered to be one of the original hosts of the African citrus psyllid, is also a host of the African form of greening.

Dodder has been used to experimentally transmit both L. africanus and L. asiaticus. *Cuscuta reflexa* has been used for citrus to citrus transmission, and *C. campestris* has been used to transmit the pathogens to periwinkle, *Catharanthus roseus* Don., with marked foliar yellowing. Liberibacter asiaticus has also been transmitted to tobacco, *Nicotiana tabacum* var. *xanthi* NC L. via dodder. Dodder itself appears to be a host to the bacteria, but in the epidemiology of the disease, dodder as a host and potential vector of the pathogen is not likely to be significant.

See the Regulatory Section for a list of regulated hosts.

Geographic Distribution

Pathogens causing citrus greening, or huanglongbing, have been reported from the following countries in Africa, Asia and South America:

Bangladesh, Bhutan, Brazil, Burundi, Cambodia, Cameroon, Central African Republic, China, Comoros, Ethiopia, Hong Kong, India, Indonesia, Japan, Kenya, Laos, Madagascar, Malawi, Malaysia, Mauritius, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Reunion, Rwanda, Saudi Arabia, Somalia, South Africa, Sri Lanka, Swaziland, Taiwan, Tanzania, Thailand, Vietnam, Yemen, and Zimbabwe.

To date, HLB pathogens have not been reported from citrus-producing regions of Australia, Mexico, countries in Central America or the Mediterranean.

SURVEY PROCEDURES

Sanitation Precautions for Inspectors

When visiting destination nurseries to conduct surveys or to take samples, regulatory officials must take strict measures to prevent contamination by plant pathogens between properties during inspections.

Although HLB cannot be transmitted by casual contact the way some citrus pest are, psyllids that vector HLB can be easily carried by contact from infected to healthy trees. Strict sanitation measures are also in place to prevent the spread of other regulated or non-regulated pests.

Wash hands with an approved microbial soap. If not using a microbial soap, wash hands with regular soap and warm water to remove soil and debris. Then use an alcohol-based antimicrobial lotion, such as Purell® (or an equivalent product with 63% ethyl alcohol). If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, an antimicrobial lotion is less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations. The citrus canker program in Florida uses GX-1027 Antimicrobial Soap (Galloway® Chemical, phone 800-445-1143) and this product can also be used to disinfect for other citrus bacterial pathogens.

Pruning shears used to cut samples should be disinfected prior to use on a new property (and preferably before use on each tree) to avoid spreading citrus exocortis or other citrus viroids. These citrus pathogens can be carried on the cutting surfaces of pruning shears, knives, and other implements used for cutting and pruning operations. Making a cut on an infected tree is sufficient to contaminate the cutting tool; subsequent cuts on other trees will introduce the viroid and infect the tree. Viroids, small pieces of "naked" RNA similar to a plant virus but lacking the protein coat, are extremely difficult to remove from the tool and are not "killed" (inactivated) by most disinfectants or even by high heat used for sterilizing other pests. A brief spray or immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (common household liquid bleach) is an effective way to inactivate citrus viroids and prevent their spread. (See Appendix 3 for more information).

General Detection Surveys

The purpose of a general detection survey is to determine if a pest is present in a defined area. This can be broad in scope, as when assessing the presence of the disease over large distances or it may be restricted to determining if a specific pest is present in a focused area. Statistically, a detection survey is not a valid tool to claim that a pest *does not* exist in an area, even if results are negative. However, negative results can be used to provide data that can be used to determine modes of dispersal, describe temporal occurrence, or determine effects of local industry practices. Compilation of even negative results that are topographically, spatially, or geographically similar can be used to develop a better understanding of the pest biology and design better control strategies in infested areas.

General survey strategies may be focused on finding the psyllid vector or finding disease symptoms, depending on the target location.

Survey Types

General detection surveys for citrus diseases are those conducted over a broad area to discover a new infestation. These surveys can be of several types, using various techniques listed below.

Symptomology Surveys: States where one of the HLB insect vectors is known to occur should concentrate visual surveys in commercial, residential, and nursery areas for symptoms of HLB after training surveyors on disease characteristics on various hosts.

Sentinel surveys*:* Survey specific designated trees on a continuing basis for other citrus diseases or fruit flies. These can be in residential or commercial groves. This type of survey was developed in Florida for the purposes of detecting new citrus canker infestations, but can be applied in the detection of mutiple diseases and insect pests of citrus. One host tree, ideally the most susceptible species, is chosen for continuous inspections every month, or every several months. By setting up a specific density of sentinel plants in areas of no known infestation and inspecting them on a regular basis, early detection is possible. The techniques in the symptomology survey are employed in sentinel surveys using visual searching for symptoms or vectors, along with trapping for vectors.

Targeted surveys*:* Sometimes referred to as “hotzone” or “demographic” surveys. Surveys are conducted in residential neighborhoods based on demographics coupled with pathway information from agency interception databases. Surveys can be concentrated where risk has been calculated with a high percentage of residents from source countries, using census data with zip-codes for defining the area. This type of survey is useful in detecting new foci of infection from, for example, unauthorized importations of infected plant material. Outreach materials can be distributed door-to-door in residential areas, where contacts are made with residents for inspections of trees on their properties.

Psyllid Vector Surveys: If the psyllid vectors (*Diaphorina citri* or *Trioza erytreae*) are not known to occur in the survey area, insect surveys should be conducted by visual inspection, sweep-netting hosts plants, or the use of sticky traps. Winged adults are easier to see than nymphs because the nymphs appear flattened on a host leaf surface similar to scale insects. Flush growth on host plants is the most likely place to find adults and nymphs. Vector surveys can be targeted in citrus nurseries and in nurseries selling the preferred host for *Diaphorina citri*, orange jasmine, *Murraya paniculata* (not a known host of HLB, the plant should not be sampled for HLB detection). In areas where the

vector is known to occurs, specimens can collected and analyzed for the presence of the HLB pathogen using molecular diagnostic techniques, however, this technique is not recommended for general detection surveys for the pathogen.

Symptomology for Inspections During Detection Surveys

Symptoms alone are not diagnostic. Other plant pathogens or cultural conditions (fertility, weather, etc.) can cause similar symptoms. Do not conclude that a plant with the symptoms described below is infected with one of the three *Candidatus* Liberibacter species. Moreover, assume that the plant is *suspect* and take samples for further testing.

HLB can be difficult to find because it does not display symptoms in its early stages (long latent period), may escape detection with current diagnostic methods, and may be vectored over a wide area before detection occurs. The normal interval between vector inoculation and symptom development ranges from 4 months, to 1 year (Hung et al., 2000).

Foliar symptoms are not specific to this disease (see Appendix 1, Figures 9-13). They can resemble symptoms produced by certain mineral deficiencies (zinc, iron, and manganese) and several other citrus diseases (blight, stubborn, and tristeza). Fruit symptoms, however, are much more characteristic of and specific to this disease, although there is some overlap with the symptoms produced by several other citrus diseases.

Foliar Symptoms: Early foliar symptoms include yellowing of leaves along the midrib and larger veins, which spread to produce a blotchy, mottled appearance (see Appendix 1, Figures 10, 13). This may not be noticed until a yellow shoot appears on the tree (see Appendix 1, Figure 9). Initially, these changes are usually confined to one limb or sector of the tree, with the “normal” limbs bearing normal leaves and fruit. However, if infected at an early age, the yellowing may spread to the entire tree. In infected sectors of trees, leaves are small, sparse, upright, and frequently have zinc deficiency symptoms including green veins with chlorotic interveinal areas, (see Appendix 1, Figure 12). Leaves with HLB have a mottled appearance that differs from nutrition-related mottling. HLB-induced mottling usually crosses leaf veins. Nutrition related mottling usually occurs between or along leaf veins. However, in both cases, leaves may be small and upright.

Fruit Symptoms: Fruit is smaller than normal and usually lopsided (caused by a curved columella). The shaded side remains green, while normal coloring develops on the other side (see Appendix 1, Figs. 13-15). The fruits are very unsaleable, with a somewhat salty, bitter taste. In contrast, fruit with similar symptoms caused by other citrus diseases is generally sweeter than normal. Seeds are generally aborted. Heavy fruit drop is also observed.

Other Symptoms: Twig dieback can occur in severe cases. Heavy leaf abscission and fruit drop, followed by out of season flushing and bloom, can occur on infected trees and branches. Young (1-2 year old) trees may die from the infection.

Traceback/ traceforward Investigations

Traceback/traceforward investigations help determine priorities for delimiting survey activities after an initial US detection. Traceback investigations attempt to determine the source of infection. Traceforward investigations attempt to define further potential dissemination through means of natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

For positive detections on homeowner properties, inquiries are conducted with the owner of the infected material to determine where it originated (nursery, from neighbors, etc.) and where it might have been further distributed.

For nursery hosts, a list of facilities associated with infected nursery stock from those testing positive for HLB will be compiled. These lists will be distributed by state to the field offices, and are not to be shared with individuals outside USDA APHIS PPQ regulatory cooperators. **Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited.**

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received HLB infected material. Speak to the growers or farm managers and obtain proper permission before entering upon private property.

Several actions need to occur immediately upon confirmation that a citrus nursery sample is positive for HLB: Check nursery records to obtain names and addresses for all sales during the prior six months. These should be grouped as (A) sales in the past month, (B) sales in the two months prior to that, and (C) sales in the three months prior to that, for a total of six months. Evaluate the disease situation, including identification and inspection of the budwood source(s) of the diseased tree(s), the location within the nursery, and the disease severity. (See the Regulatory and Control Sections for more information).

Delimiting Survey after Initial US Detection

After a new US detection, or a detection in a new area is confirmed, host surveys on commercial groves and homeowner properties in the area will be conducted. Because the disease is usually found to be randomly distributed in an infested area, all citrus trees should be inspected for the presence of HLB symptoms if possible. Be aware that symptoms may occur on only one or a few branches, or (in later stages of infection) throughout the canopy of the tree. Foliar symptoms resemble certain mineral deficiency symptoms and those

produced by several other citrus diseases.

When in doubt, always collect and submit samples for laboratory analysis. The use of a sentinel tree system like that in place for citrus canker in Florida would be an appropriate survey strategy for this disease, but a more intensive stratified survey around known positive detections will help delimit the extent of the disease in that area.

Data collection can be simplified by the use of pre-programmed hand-held units that allow ease of data recording with GPS capability. The data collected during surveys should include:

1) date of collection,
2) sample number from predetermined numbering system,
3) collector's name and agency,
4) full address including county
5) type of property, i.e., residential, nursery, commercial grove, feral or abandoned grove
6) grower's field ID numbers if appropriate,
7) GPS coordinates of the host plant and property,
8) host species, and cultivar,
9) observations of the number of infected trees,
10) the presence of vectors,
11) general conditions or any other relevant information,
12) positive or negative results from testing (recorded later).

Recording negative results in surveys is just as important as positive detections since the compiled data help define an area of infestation. A system of data collection should include an efficient tracking system for suspect samples so that their status is known during the various stages of transit and testing at authorized laboratories.

PRECAUTIONS: Before starting inspections, always determine if there have been recent pesticide applications that would make it unsafe to inspect the citrus. Check with property owners or managers for this information. Look for posted signs indicating recent pesticide applications, particularly in commercial groves.

Determine if any quarantines are in effect for the area being surveyed, such as for root weevils, citrus canker, etc. Comply with all quarantine requirements.

Before entering a new property, be sure you have permission and make certain that footwear is clean and free of soil to avoid moving soil-borne pests from one property to another. Disinfect pruning shears with bleach (see Appendix 3) to avoid spreading other citrus pathogens.

Surveys after the first US detection for that area should be most intensive around the known positive detection(s) and any others discovered through

traceback/traceforward investigations. The intensity of survey sampling around the known positives will lessen as samples are taken in concentric circles away from the positive points on a map. The level of sampling in various radii away from the known infestation will depend on recommendations from CPHST, ARS, and other scientists, as more scientific information on the biology and spread of this pest is gathered. The level of sampling will also depend on resources available. These surveys should include residential, nursery, and commercial groves with the results mapped so that potential quarantine boundaries can be determined.

Survey task forces should consist of an experienced survey specialist or plant pathologist familiar with the disease symptoms and personnel responsible for sample collection and proper recording the data and GPS coordinates.

Rapid Delimiting Survey

A method developed for doing a rapid delimiting survey is one which uses concentric annuli made of circular transects. The basic concept starts with the known positive host plant(s) in a given location. Survey task forces do inspections in increasing five mile increments along the arcs of concentric annuli.

Depending on the availability of hosts and survey resources, the first 5 mile annulus has 16 equally spaced survey points around the circle. If suspect positive hosts are discovered in the first 5 mile arc, the next survey points will be in a 10 mile annulus, with 32 points, and 15 miles with 64 points (Figure 1). If no suspect positive hosts are discovered at a five mile increment, survey crews begin to work back toward the center point to define the delimitation of the infestation.

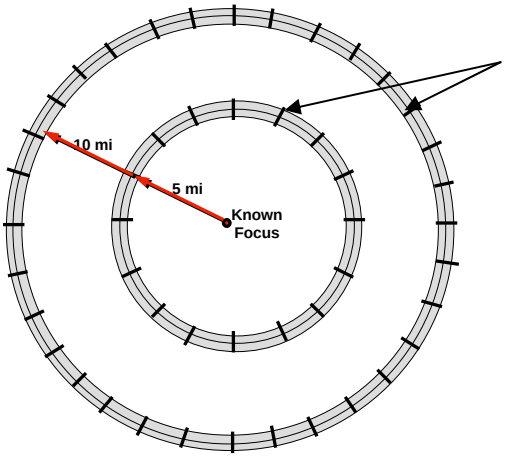


Figure 1. Sampling points along concentric annuli transects at 5 mile increments away from a known positive host tree.

Source: Tim Gottwald, ARS, Ft. Pierce, FL

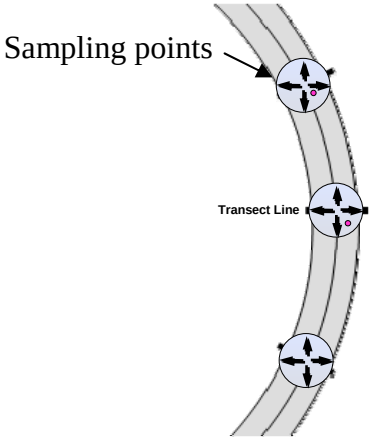


Figure 2. Sampling points along an arc transect showing where searching begins to find the nearest host tree for survey.

At each sampling point, surveyors will search for the nearest host tree in the immediate area for susceptible hosts (Figure 2). The order of preference for sampling includes 1) orange, mandarin, tangelo, and tangerine, 2) pummelo, grapefruit, and sour orange and 3) lemon and lime. Examine trees for the presence of yellow shoots, foliar mottling, zinc pattern deficiency, and yellow viens (see Appendix 1 for symptomology).

If not disease symptoms are found or no hosts are found in the sampling point area, search for hosts and symptoms at points adjacent on the same annulus. Depending on the survey crew's instructions, and if no evidence of the disease is found, searches can begin in 1 mile increments back toward the center of the sampling annuli following the same procedure as above (Figure 3).

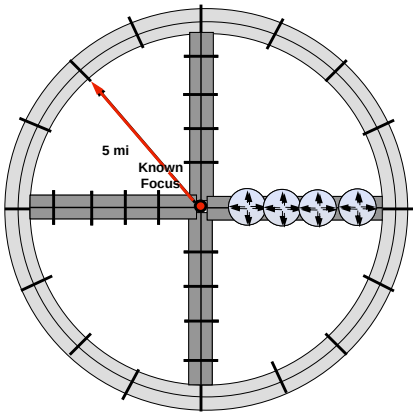


Figure 3. If the disease is not found in the transect sampling point, travel back toward center sampling in one mile increments.
Source: Tim Gottwald, ARS, Ft. Pierce, FL

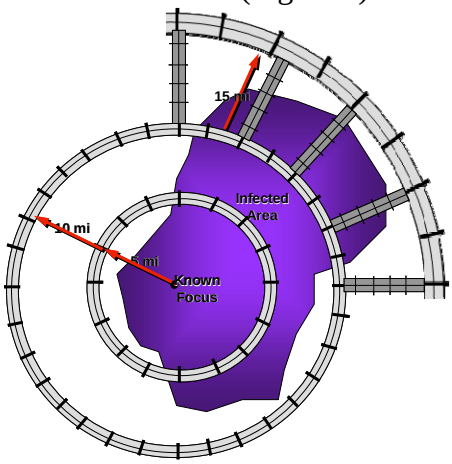


Figure 4. If disease is found to extend beyond an annulus in one or two directions, use a partial annulus combined with information from other transects to define the boundaries of the infestation.

Further narrowing of infestation boundaries is possible by continuing sampling in different directions along newly defined transects along the annuli. Once suspect samples have been identified and new positive locations are mapped, the points around the original positive trees will more clearly define the extent of the infestation. Refinements will occur with increased sampling.

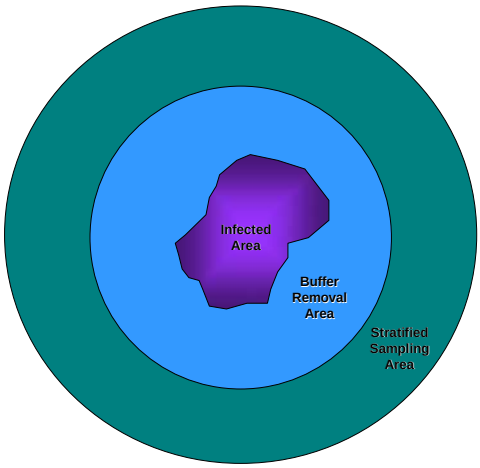
Depending on the configuration of the survey area, linear transects can be used instead of concentric annuli for conducting these kinds of surveys. For example, urban and suburban areas may be more effectively surveyed using linear transects.

Stratified Surveys Over Large Growing Areas

After one or more infestations are delimited, regulatory and control measures may require the removal of exposed hosts around the known infested areas. Further surveys are necessary to discover satellite infestations or other areas of potential infection. A sentinel or other stratified survey will be designed to accomplish this.

Figure 5. Diagram showing a defined infected area after a delimiting survey, buffer of exposed hosts removed, and area outside that area where other stratified surveys are conducted.

Source: Tim Gottwald, ARS, Ft. Pierce, FL



Vector Surveys for Predicting Future Outbreaks

Citrus psyllids that are in a known area of HLB infestation may be subjected to PCR diagnostics to determine their status as carriers of the pathogen. See the Diagnostics Appendix 5 for methodology, submission of psyllid vectors for identification. Psyllids can be collected with aspirators, sweep-nets, or general detection traps. Collection of psyllids for this purpose should include all appropriate data as plant samples. These kinds of vector surveys might be best conducted on the edges of citrus groves since it appears in some cases that psyllid populations are higher there. Surveyor conducting these kinds of surveys should be aware that the incidence of vector infection is directly proportional to the disease incidence in an area. Negative pathogen detections in vectors should not be interpreted to mean the non-occurrence of disease in the area.

In areas of known disease occurrence, results from a sufficient number of vector samples are overlaid in map layers with known plant infections and citrus occurrence. Analysis of these maps may help predict where HLB may show up in subsequent seasons.

Guidelines for Residential Surveys

Inspect citrus trees on the property for HLB symptoms and citrus psyllid presence. Examine newer foliage and branches on larger trees, as older foliage frequently shows symptoms of other pathogens, covered with sooty mold, etc., which may make it difficult to see HLB symptoms. If symptoms are found on a tree, samples, consisting of leaves (with petioles attached) and green twigs 6-8 inches long, should be cut from those areas of suspect trees that show the best symptoms. Some asymptomatic leaves on the same branches may also be submitted with symptomatic ones. If you have access to a camera, take a photograph of the symptomatic region of the tree. Also, take photographs of the entire tree being sure to include the symptomatic region in the photograph.

Samples should be placed in zip-lock plastic bags (place the leaves between dry paper towels and bundle the twigs together with a rubber band), along with their identifying numbers. Double bag the sample and place the sample information

on a sheet in the outside bag.

Since fruit symptoms are far more indicative of HLB than foliar symptoms, you should carefully examine any fruit that may be present on the affected branches. If any fruit showing HLB symptoms are found, several typical symptomatic fruit should be submitted with the leaf and twig samples. Fruit is not known to harbor high concentrations of the bacterium, so it should not be sampled without accompanying leaf or stem tissue from the same tree. Fruit samples should be wrapped in dry paper towels and placed in paper bags, along with their identifying numbers. Double bag the sample.

Keep the samples as cool as possible, and ship them via overnight delivery to the laboratory. Call the laboratory and advise them that samples are being shipped. Mark the tree with the sample identification number and draw a map of its location on the property and record GPS coordinates, as tags may occasionally disappear.

If most (or all) citrus trees on the property show symptoms throughout the tree, it may indicate a mineral deficiency problem. Submit samples from several of the trees with the most "typical" symptoms. Any trees with symptoms confined to one or a few branches, rather than the entire tree, should be sampled.

Trees should also be checked for the presence of psyllid vectors, particularly if new growth is present. If small pit galls are seen on young leaves, African citrus psyllids (*Trioza erytreae*) may be present. Samples of this psyllid should be collected and submitted for identification, regardless of which life stage the African psyllids are found. Mark the tree the psyllids were collected from and map its location using GPS.

Note the presence or absence of orange jasmine (*Murraya paniculata*) on the property, and if present check for Asian citrus psyllids. If Asian citrus psyllids (*Diaphorina citri*) are found in any states other than Florida, Hawaii, or Texas, samples of these psyllids should be should be collected and submitted for identification as outlined in Appendix 2 and tested for the presence of Liberibacter as outlined in Appendix 5.

Guidelines for Commercial Grove Survey

IMPORTANT: Reschedule inspections for commercial grove blocks when harvesting operations are underway. Ask the owner or manager if any other cultural operations (e.g., hedging, irrigating, or fertilizing) are planned that might interfere with the inspection, and reschedule if necessary. Surveys should be scheduled for times when the citrus orchards are producing new foliage. Determine when the most likely season for flush of new leaves occurs in your area and try to target that time for surveys. Also check all sentinel trees in the areas adjacent to commercial groves

It will be necessary to walk every row in the grove in order to inspect as much of the newer growth and young branches on the outside of the trees as possible. Older foliage towards the inside of larger trees frequently show symptoms of other pathogens, covered with sooty mold, etc., which may make it difficult to see HLB symptoms, and should not be inspected. If symptoms are found on a tree, samples, consisting of leaves (with petioles attached) and green twigs 6-8 inches long, should be cut from those areas of suspect trees that show the best symptoms. Some asymptomatic leaves on the same branches may also be submitted with symptomatic ones.

Leaf and twig samples should be placed in zip-lock plastic bags (place the leaves between dry paper towels and bundle the twigs together with a rubber band), along with their identifying numbers and accurate GPS coordinates. Double bag the sample.

Since fruit symptoms are far more indicative of HLB than foliar symptoms, you should carefully examine any fruit that may be present on the affected branches. If any fruit showing HLB symptoms are found, several typical symptomatic fruit should be submitted with the leaf and twig samples. Fruit is not known to harbor high concentrations of the bacterium, so should not be sampled without accompanying leaf or stem tissue from the same tree. Fruit samples should be wrapped in dry paper towels and placed in paper bags, along with their identifying numbers. Double bag the sample and include collection information on a sheet inside the bag.

Keep the samples as cool as possible, and ship them via overnight delivery to the authorized laboratory. Before shipment, call the laboratory and advise them that samples are to be expected. Mark the tree with the sample identification number and draw a map of its location within the block and the location of the block on the property. It may be helpful to designate one corner of the block, e.g., the NE corner, and count the number of rows from that corner and the number of trees down the designated row to precisely locate the sampled tree, or record the appropriate GPS coordinates. Flagging the sampled branch or branches will make it easier for subsequent sampling.

Trees should also be checked for the presence of psyllids, particularly if new growth is present. If small pit galls are seen on young leaves, African citrus psyllids (*Trioza erytreae*) may be present. Samples of this psyllid should be collected and submitted for identification, as outlined in Appendix 2 regardless of which life stage suspect African psyllids are found in. Mark the tree the psyllids were collected from and map its location. If Asian citrus psyllids (*Diaphorina citri*) are found in any states other than Florida or Texas, samples should be collected and submitted for identification as outlined in Appendix 2. Please note the position of any sample with GPS coordinates.

Guidelines for Nursery Survey

Citrus nurseries generally employ one or more of the following operational procedures: 1) trees grown outdoors in rows in the ground, 2) trees grown outdoors in containers (may be under shade cloth or in a lath house), 3) trees grown in a fully-enclosed screenhouse, generally in containers, and 4) trees grown in a greenhouse, generally in containers. Trees lined out in rows in the ground are usually budded sequentially by budders moving down the rows, whereas this is often not true of container-grown trees, which are moved about as nursery operations dictate. **NOTE:** Nursery stock may be widely disseminated throughout a large geographic area, especially stock purchased by big retail chains, making it very important to thoroughly inspect **all** host trees being grown in the nursery.

Depending on tree and row spacing, it may be necessary to walk every row in order to see all of the foliage on each tree. Examine newer foliage and branches on larger trees, as older foliage is frequently shows symptoms of other pathogens which may make it difficult to see HLB symptoms. Any budwood source trees located at the nursery should also be inspected. **IMPORTANT:** pruning shears used to cut samples from budwood source trees should be sanitized prior to use to avoid spreading citrus viroids such as exocortis (see Appendix 3).

Samples, consisting of leaves (with petioles attached) and green twigs 6-8 inches long, should be cut from those areas of suspect trees that show the best symptoms. Samples should be placed in plastic bags (place the leaves between dry paper towels and bundle the twigs together with a rubber band), along with their identifying numbers and accurate GPS coordinates. Double bag the sample.

Since fruit symptoms are far more indicative of HLB than foliar symptoms, you should carefully examine any fruit that may be present on the affected branches. If any fruit showing HLB symptoms are found, several typical symptomatic fruit should be submitted with the leaf and twig samples. Fruit is not known to harbor high concentrations of the bacterium, so should not be sampled without accompanying leaf or stem tissue from the same tree. Fruit samples should be wrapped in dry paper towels and placed in paper bags, along with their identifying numbers along with accurate GPS coordinates. Double bag the sample.

Flag the sampled tree and attach the appropriate identifying numbers to it, as well as mapping its location within the block and the nursery and acquire accurate GPS coordinates. Keep the samples as cool as possible, and ship them via overnight delivery to the authorized laboratory. Mark the samples and the package to indicate that they are nursery samples. A hold will be placed on the nursery until the samples are processed; it is important to clearly mark the package so that the laboratory can give these samples priority handling. If high suspect samples are obtained, instruct the nursery owner or manager not to move

nursery stock out of or within the property until results are obtained. Call the laboratory and advise them that nursery samples are being shipped.

Several suspect trees occurring sequentially in a row of young field-grown stock suggests the possibility that they were propagated from infected budwood. Question nursery personnel to determine the source of the budwood used to bud the stock and examine the tree if present. **IMPORTANT:** pruning shears used to cut samples from budwood source trees should be sanitized prior to use on each tree to avoid spreading other citrus diseases (see Appendix 3).

Trees should also be checked for the presence of psyllids. If small pit galls are seen on young leaves, African citrus psyllids (*Trioza erytreae*) may be present. Samples of the psyllids should be collected and submitted for identification, as outlined in Appendix 2. Mark the tree from which the psyllids were collected and map its location and obtain accurate GPS coordinates. Note the presence o absence of orange jasmine (*Murraya paniculata*) on the property, and if present check for Asian citrus psyllids. If Asian citrus psyllids (*Diaphorina citri*) are found in any states other than Florida or Texas, samples should be collected and submitted for identification as outlined in Appendix 2.

Other Hosts Surveys

Inspect and sample other ornamental and native hosts (see Regulatory Section) in the area for symptoms. These plants may serve as reservoirs for the pathogen.

Monitoring Surveys

Inspectional visits during the same, or subsequent, growing seasons is appropriate in order to examine hosts for symptoms. After any control or eradication procedures are conducted, it is necessary to do follow-up monitoring surveys to assess the success of the program. Also, in areas where control actions have occurred and trees have been removed, monitor for new host plants that may have sprouted from roots remaining in the ground.

DIAGNOSTICS AND IDENTIFICATION

Importance

Accurate identification of this quarantine pest is pivotal to assessing its potential risk, developing a survey strategy, and deciding the level and manner of control. Consult Appendix 5 for details on approved diagnostic methods for this pathogen.

Authorities

A USDA-recognized national authority for the quarantine taxon must positively identify the suspected pest before initiation of any program quarantine activities. Confirmatory molecular testing using PCR for the pathogens that cause citrus greening is performed at the USDA, APHIS, PPQ National Plant Germplasm and Biotechnology Laboratory (NPGBL) in Beltsville, Maryland. This laboratory also has all the necessary permits and containment approvals to handle select agents.

In the future, other laboratories may obtain certification and registrations for making presumptive positive determinations but must abide by guidelines set under various permit and authorizations maintained by APHIS Plant Protection and Quarantine.

PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring select agents are required to be a registered as a select agent laboratory. However, diagnostic laboratories that identify select agents are exempt from this requirement as long as they complete an APHIS/CDC Form 4 and destroy or transfer infected material to a laboratory registered with the APHIS Select Agent Program within the mandatory 7 days.

The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status. If any material is shipped interstate, it is a requirement that the receiving laboratory has a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services on (301) 734-8758.

Federal regulation on Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents and toxins. Once an

unregistered diagnostic laboratory identifies a select agent, they must immediately notify the APHIS Agriculture Select Agent Program, complete an APHIS/CDC Form 4 and submit within 24 hours, and either destroy or transfer the agent to a registered entity within 7 days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample for voucher purposes and that sample forwarded to the USDA Beltsville Laboratory came back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. If the determination of the unregistered laboratory is to destroy the sample, this must take place within seven (7) days of results notification and a PPQ Officer must witness the destruction of the sample on or before the 7-day period expires. Clarification of this and other information related to adherence to the select agent regulations is available on the following APHIS website: http://www.aphis.usda.gov/programs/ag_selectagent/index.html, or call (301) 734-5960.

Centralized National Survey Screening Laboratory

After the initial US detection of Huanglongbing (HLB, or citrus greening), a national survey conducted in citrus growing states requires consistency in diagnostic procedures and assurances that requirements for handling select agents are followed. Under a USDA national survey funded program, centralized molecular diagnostic screening tests are to be conducted for all high and medium suspect HLB host material encountered during surveys. This laboratory will then forward any suspect positive samples to the NPGBL in Beltsville, MD for confirmatory testing.

Until an HLB proficiency test panel and laboratory approval program is implemented, all suspect HLB plant and vector samples collected in the APHIS funded national survey will be forwarded to the following laboratory approved to perform molecular diagnostic screening for this program:

Attention: Michael Sussman, Ph.D., Molecular Biologist
USDA-AMS Science and Technology Programs
National Science Laboratory - Molecular Biology Section
801 Summit Crossing Place, Suite B
Gastonia, NC 28054

Michael.Sussman@usda.gov
704-867-3873 voice 704-853-2800 fax

Mark all packaging for all HLB Survey samples:
"*ATTENTION, APHIS Citrus Samples*"

Classification of Plant Sample Symptoms

Samples of plants with symptoms taken by field personnel must be first submitted through their normal regulatory networks. These labs will perform diagnostics to screen out symptomatic samples of other causes (Consult Appendix 1 for symptomology examples).

Forward to the USDA-AMS Gastonia laboratory all **High-** and **Medium-suspect** leaf and stem samples that are collected as a part of the national survey. (Do not forward fruit samples without accompanying suspect leaf samples from the same tree).

The class of samples are categorized by the following characteristics:

HIGH suspect samples: Classic HLB mottle alone or accompanied by one or more of the following:

- zinc-like deficiency
- yellow veins
- corking veins
- misshapen or oddly colored fruit

*** Classic HLB mottle is usually visible on both leaf surfaces and mottling/discoloration passes through veins.*

MEDIUM suspect samples: Non-classical mottle alone or in combination with the following symptoms:

- yellow veins
- vein corking
- chlorotic leaves
- zinc deficiency

*** Non-classical mottle visible only on adaxial surface and may or may not cross veins.*

LOW suspect samples:

- Zinc and other general deficiencies
- Mottling resulting from insect injury, fungal diseases, and mechanical damage to leaves
- Naturally senescing leaves
- Genetic variegation

Sample Storage and Forwarding

Leaf samples are preferred for molecular diagnostic testing since the diagnostic test is conducted on leaf mid-ribs. Leaves that are attached to stems are desirable. A minimum of 20 leaves should be sent if possible to complete all diagnostic testing and insure enough material will be forwarded if federal confirmation is required.

Place leaf and stem samples with paper towels in double zip-lock bags and express as much of the air out as possible prior to sealing the bag. Include dry paper towels in the bag with the sample so they can absorb moisture that will cause plant material to degrade. Although fruit is not the most desirable sample, if symptomatic fruit is sent, place in double paper bags. Keep leaf and fruit samples cool, but not frozen, preferably in an ice chest, while transferring them and waiting for preparation for mailing to the screening laboratory. Write the sample ID number legibly using a Sharpie® pen on the zip-lock bag.

Psyllid Samples

If citrus psyllids are forwarded for detection of the citrus greening pathogens, make sure the insects are first correctly identified as citrus psyllids. Place them in a leak-proof vial with 95% ethyl alcohol. The alcohol concentration is important for effective PCR analysis. Write the sample ID number in pencil on a label inserted into the vial. No less than 5 insects must be sent, and more are preferable.

If the psyllid vectors of HLB (*Diaphorina citri* or *Trioza erytreae*) are not known to occur in the state, and suspect detection is made, fill out a separate PPQ form 391 marked "Urgent" and forward to the following address for determination (see Appendix 2 for more information):

Leader, Taxonomic Services Unit,
USDA, ARS, BA, PSI
Building 046, Room 101A, BARC-EAST
Beltsville, MD 20705-2350

Sample Packaging and Documentation

Samples must be sent by overnight delivery (FedEx® is preferred), and on the same day they are collected if at all possible, or before 12:00 noon the following day. Ice packs are not needed or recommended. Packaging of all samples should be in a larger zip-lock bag made leak proof, then must be placed in a sturdy cardboard outer box with insulation to prevent movement within the box during shipping. Include the completed PPQ form 391, and any relevant tags or barcodes that came with the sample.

Only send samples by overnight delivery on Monday through Thursday. Saturday delivery will not be accepted unless special arrangements with the USDA-AMS Gastonia laboratory are made prior to shipping.

Sample Labeling, Numbering, and Record Keeping

An electronic data collection system for survey and sample collection is currently being developed. Until the protocols for that system are finalized, complete a PPQ form 391 (*Specimens for Determination*) for each sample.

The submitter should complete and include a hard copy of the PPQ form 391 inside the outside bag of double-bagged samples. Assign and record for each sample a unique ID sample number with a predetermined format. Assure that the sample is linked to any survey data collected for that sample by including the Survey ID number on the form. This will enable the linkage of the sample to all the field collection information.

In block 1 of the PPQ form 391, enter and label the: assigned sample ID number first with the first two letters designating the state two letter code. Also enter the survey ID in parenthesis. The state's own lab sample accession number can also be added for record keeping. Use the following format:

Sample ID # XX-00000 (Survey ID # _____)

In the remarks section (block 22), give the name of the office or diagnostic laboratory forwarding the sample, plus a contact name, e-mail address, and phone number of the contact. Include the date forwarded to USDA-AMS Gastonia lab.

In block 23, enter the preliminary diagnosis (e.g., "High suspect HLB", "Medium suspect HLB").

Inspectors must provide all relevant collection information with samples. This information should be communicated within a State and with the regional office program contact. If a sample tracking database is available at the time of the detection, please enter collection information in the system as soon as possible.

Centralized National Survey Screening Laboratory Results

The USDA-AMS Gastonia diagnostic screening laboratory normally has results within 24 hours after receiving samples. If a presumptive positive sample is determined by PCR tests, the sample will be forwarded to the NPGBL with the originating state and PPQ officials being notified.

Web-based Reporting Tool

A web-based survey and diagnostic data collection and reporting tool are in the development phase for an HLB program. The guidelines for data entry, access, and reporting are provided separately by the PPQ Regional Program Manager.

State or other Diagnostic Screening Laboratories Results

If a state, university, NPDN, or private laboratory performs a PCR assay and detects a presumptive positive outside the national survey process outlined here, they must abide by the requirements under the Agriculture Bioterrorism Protection Act of 2002 (7 CFR 331).

Diagnostic screening laboratories receiving samples are to communicate the date of receipt with their State Plant Regulatory Official and/or State Plant Health Director. All relevant sample information and the diagnostic lab's determinations must be communicated as soon as possible within a State and with the PPQ regional office program contact.

Approved Laboratory for Confirmatory Testing

Once the plant material has been screened and is known to be presumptive positive by molecular diagnostics, **as soon as possible by overnight carrier, the screening forward the sample with the accompanying PPQ form 391 as soon to the CPHST NPGBL in Beltsville, MD for confirmation**. The NPGBL is authorized by APHIS to receive suspect domestic select agent plant pathogens under APHIS permit number 65253. A copy of the NPGBL permit for suspect domestic select agent pathogens need not accompany the package.

Potentially Actionable Suspect (PASS) samples in a program must be confirmed by the CPHST NPGBL which is the APHIS PPQ National Identification Services recognized taxonomic authority for this pathogen. In the case of HLB, the first presumptive positive(s) in a state are PASS samples. Presumptive positives in counties outside of the initial positive county are also PASS samples. Any presumptive positive from a new host or other unexpected or unusual find must be treated as PASS samples.

The diagnostic laboratory, with proper authorizations to do final confirmatory testing for *Candidatus* Liberibacter is the USDA, APHIS, PPQ-CPHST NPGBL in Beltsville, MD. (*address below*).

Attention: Dr. Laurene Levy / Renee DeVries
USDA, APHIS, PPQ-CPHST, NPGBL
BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705
phone number: 301-504-7100
fax number: 301-504-8539

Please notify the NPGBL by email or fax that material is being sent by sending an email with the overnight service name and tracking number (notify laurene.levy@aphis.usda.gov and renee.m.devries@aphis.usda.gov).

After a period during the establishment of an emergency program, other laboratories may be certified and given authorizations to perform APHIS PPQ diagnostic tests.

Completing the PPQ form 391 Determination Section

Diagnostic screening laboratories must write their determinations for each sample on the PPQ form 391 that came with the sample. Include the name and phone number of the responsible diagnostician, keep a copy, and follow the same sample packaging instructions as above.

The APHIS Beltsville staff requests the following additional information please be noted-on the 391 form:

a) What specific plant part tested positive? (for example for HLB that should be the midrib.)

b) What method of PCR and what method of DNA extraction if by chance different from the APHIS authorized protocols were used.

c) Labeled photographs of the PCR test that can be emailed to Dr. Levy at the NPGBL while the plant material is in route. However these photographs must arrive prior to NPGBL testing. (laurene.levy@aphis.usda.gov and wenbin.li@aphis.usda.gov)

d) For high suspect samples, forwarding photographs of associated symptomatic fruit and foliage is extremely helpful.

Saturday Delivery to NPGBL

Normally, it is not recommended that samples be sent on Thursdays or Fridays because of the possibility of their deterioration occurring over the weekend. Depending on need and when approved by APHIS officials, samples may be sent on Thursdays or Fridays by FedEx® because it is possible to have Saturday delivery by overnight carriers to the Beltsville facility. **However this must be determined by consultation and arrangement with APHIS and state officials prior to assuming that the laboratory will be operating on Saturday**. If you verify with APHIS officials that samples will be accepted on Saturday, the FedEx® tracking number to the NPGBL in Beltsville must be provided by Friday no later than 2 PM EST by email so they can notify their Fedex® local office to authorize Saturday delivery**.**

Notification of State Officials of Sample Submissions and Results

Notify the State Plant Health Director and State Plant Regulatory Officials in the sample state of origin and fax the PPQ regional office of any sample forwarding information, completed documentation, including overnight freight tracking information. Once results are known, States will be notified by the PPQ regional office of the results.

Do not contact the NPGBL Beltsville to get sample results. This information will be reported through the appropriate and approved reporting lines to the regions and States from PPQ headquarters as soon as they are available. The NPGBL will direct any inquiries to PPQ headquarters.

Sample Processing Time

Growers and cooperators need to be aware that sample processing and testing time of at least 48 hours is required for the NPGBL. This is in addition to the time it takes to process and forward samples from the intermediate state or cooperating university diagnostic laboratories.

REGULATORY PROCEDURES

Instructions To Officers

Agricultural officers must follow instructions for regulatory control measures, treatments or other procedures when authorizing the movement of regulated articles. A full understanding of the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatments may be used in accordance with labeling restrictions. During all field visits, please ensure that proper sanitation procedures are followed as outlined in the Survey section.

Quarantine Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification (PPQ form 523) may be issued to hold articles or facilities, pending positive identification by a USDA, APHIS, PPQ recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register.*

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides for authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under state authority. State departments of agriculture normally work in conjunction with federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the measures taken by the state are inadequate, USDA can take intrastate regulatory action provided that the governor of the state has been consulted and a notice has been published in the *Federal Register*. If intrastate action cannot or will not be taken by a state, the PPQ may find it necessary to quarantine an entire state.

PPQ works in conjunction with state departments of agriculture to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property in the absence of owner permission. PPQ prefers to work with the state to facilitate access when permission is denied, however each state government has varying authorities regarding entering private property. A General Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas where PPQ and the state department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or

State Plant Regulatory Official (SPRO) in the affected state.

Overview of Regulatory Program for HLB after a US Detection

Once an initial US detection is confirmed, holds will be placed on the property by the issuance of an EAN. Immediately place a hold on the property to prevent the removal of any greening host plants, budwood, or psyllid vector hosts. This should include both citrus and non-citrus host plants, such as orange jasmine, *Murraya paniculata*. There is no need to place a hold on fruit, as it poses no risk of pathogen movement to other trees, provided care is taken to prevent inadvertently moving psyllid vectors with the fruit.

Traceback/traceforward investigations from the property will determine the need for subsequent holds for testing and/or taking further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures prescribed.

Record Keeping

Record keeping and documentation is important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by homeowners, the grower, farm manager or nursery manager for where plant material was shipped, how plant material may have moved within the facility, and any cultural or sanitation practices employed.

Keep a detailed accounting of the numbers and types of plant material held, destroyed and/or requiring treatments in control actions. Consult a master list of properties distributed with the lists of suspect nurseries based on traceback/traceforward investigations, or nurseries within a quarantine area. Draw maps of the facility layout to located suspect plants, other potentially infected areas. When appropriate, take photographs of the tree symptoms property layout, document plant propagation methods, labeling, and any other situation that may be useful for further investigations and analysis.

Keep all written records filed with Emergency Action Notification (EAN, PPQ form 523) copies, including copies of sample submission forms, documentation of control activities, and related State issued documents if available.

Issuing an Emergency Action Notification

An EAN is issued to hold all host plant material at facilities that have the suspect plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not suspect or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision to destroy

plants is made, or in the case of submitted samples, once positive confirmation is received, the same EAN for which the plants are on hold is used to also document any actions taken such as destruction and disinfection. Additional quarantine action may be warranted in the case of groves testing positive for HLB pathogens.

If plant lots or shipments are to be held as separate units, it is advisable to issue separate EAN's for each held unit of suspect plant material associated with that unit. EAN's are issued under the authority of the Plant Protection Act of 2000 (statute 7 USC 7701-7758). It is advised that States issue their own hold orders parallel to the EAN to ensure that plant material cannot move intrastate.

When using EAN's to hold articles, it is most important that the EAN language clearly specify the actions to be taken. An EAN issued for positive testing and positive associated plant material must clearly state that the material must be disposed of, or destroyed, and areas disinfected. Include language that these actions will take place at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, then be sure to document on the same EAN, any disposal, destruction, and disinfection orders resulting from investigations or testing.

For Block 1, enter the name of location of the nearest PPQ office. Under "Name of Article" in block 3, enter the host scientific name and cultivar.". In Block 4, enter the property address, nursery, or grove number or name or other information indicating the location of the plant material held. In the Shipper Block 6, enter the plant material source if known. Blocks 7 and 8 can be left blank unless that information is known.

To place plant material on a property on "Hold", in Block 12 of the EAN, enter for the Pest : "*Candidatus* Liberbacter asiaticus" or the correct name of a different HLB form. The authority under which actions are taken is The Plant Protection Act of 2000, Statute 7 USC 7701-7758. In block 15, the Action Required with suggested text as follows:

"All host plants of the *Candidatus* Liberbacter asiaticus pathogen and psyllid hosts are prohibited from movement from the property pending further notification by USDA APHIS PPQ and/or the State department of agriculture. No other disease host material, or host material of the insect, *Diaphorina citri*, may leave the property until further evaluations can be made. After further investigations are conducted, the listed plants and other host material, if a positive detection is confirmed on the property, will be treated/destroyed under supervision, with approved methods in accordance with USDA and state policies. Any additional hosts the insect vector on the property are subject to federal and State quarantine requirements prior to movement from the property"

Regulated Articles

Once initial detections are confirmed in an area (positive county or predetermined buffer area around positive finds after a thorough delimiting survey), regulated articles include all live host plant material in that area.

HLB pathogens are spread by vectors and fresh propagative plant material harboring the bacteria within the phloem of the plant tissue. Whole live plants, stems, leaves, and cuttings are regulated.

Fruit and seed are currently not considered a pathway for HLB disease spread and so are **not** regulated. However, precautionary fruit cleaning and/or inspections should be performed in areas where the psyllid vector occurs since the fruit may be a pathway for hitchhiking citrus psyllids.

The hosts of *Candidatus* Liberberbacter listed in Table 1 are prohibited movement outside the quarantine area. If one of the psyllid vectors has been confirmed in the state, movement of the pysllid host plants listed in Table 2 are also restricted and subject to regulatory treatments.

Table 1. Regulated hosts of *Candidatus* Liberibacter

Huanglongbing Hosts

Scientific name	**Common name**
Aeglopsis chevalieri	Chevalier's aeglopsis
Balsamocitrus dawei	Uganda powder-flask
Calodendrum capensis	Cape chestnut
X *Citrofortunella microcarpa*	calamondin
X Citroncirus webberi	citrange
Citrus spp.	sweet orange**, mandarine orange**, sour orange*, lemon*, grapefruit*, tangerine, pomelo*,etc.
Clausena indica	clausena
Clausena lansium	wampee, wampi
Fortunella spp.	kumquat
Limonia acidissima	Indian wood-apple
Microcitrus australasica	finger-lime
Murraya koenigii	curry-leaf
Poncirus trifoliata	trifoliate orange
Severinia buxifolia	Chinese box-orange
Swinglea glutinosa	tabog
Toddalia lanceolata	toddalia
Triphasia trifolia	trifoliate lime-berry

**Highly susceptible
*Moderately susceptible

Citrus Psyllid Hosts

The host list above are all potentially hosts for the psyllid vector as well as HLB, however a number of citrus relatives are also hosts for the psyllid, but have not been shown to be hosts for the disease. They are important as reservoirs or potential pathways for infected insect vectors to non-infested citrus growing states and should be regulated as such.

Table 2. Host of the Asian citrus psyllid *Diaphoria citri* and not *Candidatus* Liberbacter.

Scientific Name	**Common Name**
Aegle marmelos	bael, Bengal quince
Afraegle gabonensis	Gabon powder-flask
Afraegle paniculata	Nigerian powder-flask
Atalantia sp.	atalantia
Citropsis gilletiana	Gillet's cherry-orange
Citropsis schweinfurthii	African cherry-orange
Clausena anisum-olens	anis
Clausena excavata	clausena
Eremocitrus glauca	Australian desert-lime
Eremocitrus hybrid	desert-lime
Merrillia caloxylon	flowering merrillia
Microcitrus australis	Australian round-lime
Microcitrus papuana	desert-lime
X Microcitronella 'Sydney'	faustrimedin
Murraya paniculata *	*orange jasmine
Naringi crenulata	naringi
Pamburus missionis	pamburus
Toddalia asiatica	orange-climber
Vepris lanceolata	white ironwood
Zanthoxylum fagara	wild-lime

*preferred psyllid host

Shipment of Insect Vectors Hosts

Nurseries in states with confirmed detections of either citrus psyllid vector species that wish to ship any insect vector hosts (not hosts of HLB) from a regulated property or quarantined area to non-citrus growing states must do so under a limited permit and a compliance agreement that requires prescribed regulatory treatments listed below, to eliminate the risk of spreading live insect vectors. Citrus growing states without established HLB infections normally will be prohibited destinations for psyllid hosts regardless of treatments applied.

Regulatory Treatments for Psyllid Hosts Nurserystock

In citrus growing states where the citrus psyllid is known to occur, within HLB positive quarantined counties, or within established buffer areas around positive tree finds, the psyllid host plants in Table 2 are eligible to move out of a quarantine area after regulatory treatments are applied.

These treatments will be a drench with a labeled efficacious systemic insecticide followed by a foliar spray at specified time periods prior to shipment. All articles regulated as psyllid hosts must be treated with a drench containing imidacloprid as the active ingredient and receive a foliar spray with a product containing either acetamiprid, chlorpyrifos, or fenpropathrin, as the active ingredient.

For example, all plants stated above are treated 30 days prior to movement with:

Marathon ® (3125-492-59807, 60 WP, follow label rate, drench)

And, in addition, 10 days prior to movement, the plants will receive treatment with:

> Tame ® (59639-77 2.4, EC, follow label rate, foliar spray) **OR**
> Dursban ® (655-499 4, E, follow label rate, foliar spray), **OR**
> Discus® (432-1392-59807, follow label rate, foliar spray), **OR**
> Tristar ® (8033-22-1001, , follow label rate, foliar spray).

The treatments will be followed by a visual inspection for living psyllids within 72 hours prior to certification and shipping.

See Appendix 6 for information on labeling and products available.

Quarantine Area

In states without confirmed presence of one of the citrus psyllid vectors regulatory officials, in consultation with an HLB science panel should consider a buffer distance around known positive detections in which to regulate nursery stock.

In states with confirmed psyllid vector presence, once a positive disease detection is confirmed, all nurseries with disease and psyllid host material within an agreed upon buffer area are subject to quarantine and adherence with the prohibition of shipping HLB hosts (Table 1) outside the quarantined area and the psyllid hosts (Table 2) without required regulatory treatments.

Grower Requirements Under Quarantine

Depending upon decisions made by Federal and State regulatory officials in consultation with an HLB science panel, quarantine areas may have certain other requirements for groves, nurseries, or residential properties in that area, such as tree removal and destruction, psyllid control measures, or plant waste material disposal.

Any insecticides used to control psyllids or herbicides used to treat plants will be labeled for that use or exemptions will be in place to allow the use of other materials.

Nurseries within quarantine areas and other at risk areas are required to screen all outdoor host plant propagation areas to keep areas free of psyllid vectors.

Establishing a Federal Quarantine

Regulatory actions undertaken using EAN's continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfection orders or longer term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers, and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing, and risk assessment will determine the area to be designated for a federal, and parallel state quarantine. Risk factors will take into account positive testing, positive associated, and potentially infested exposed trees. Boundaries drawn may include a buffer area determined based on risk factors and epidemiology.

PPQ may issue a Federal Order, followed by an interim rule establishing a quarantine to be published in the Federal Register which is normally drafted by PPQ headquarters staff in consultation with the region, SPHD, and SPRO. The conditions that growers must abide by within a quarantine area are included in the rule. Regulated articles and conditions allowing movement of articles out of the regulated area are determined and included in the regulation, along with other administrative requirements.

Removing Areas from Quarantine

If investigations determine the quarantine restrictions on fields are adhered to over the prescribed time periods, actions are documented, fields can be released from quarantine restrictions. Notify growers that their fields may be subject to additional monitoring by State or Federal officials for the presence of HLB pathogens.

Regulatory Records

Maintain standardized regulatory records and database(s) in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

Use of Chemicals

The PPQ Treatment Manual and this Guideline identify the authorized chemicals, and describe the methods and rates of application, and any special application instructions. See the Control section for more information. Concurrence by PPQ is necessary before using any other chemical or procedure for regulatory purposes.

CONTROL PROCEDURES

Overview

Plant Protection and Quarantine develops and makes control measures available to involved states. Environmental Protection Agency (EPA) approved and labeled treatments will be recommended when available. If additional treatments selected are not labeled for use against the organism or in a particular environment, an emergency exemption can be requested and obtained under Section 18, or 24(c), special local need (SLN), of FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act), as amended.

The Citrus Health Response Plan

The Citrus Health Response Plan in development in 2006 in Florida will have recommendations and a regulatory component that includes long-term management practices for HLB while maintaining production and commerce. The procedures developed as a part of that process will provide further guidelines on HLB disease management.

Control Decisions and Oversight

All quarantine actions related to destruction are to be witnessed, supervised, and documented by a federal and/or state plant regulatory official whenever possible. Because some strains of citrus greening are listed as select agents under the Agriculture Bioterrorism Protection Act of 2000, proper supervision and documentation of destructions of infected plant material is critical. If a PPQ representative is not available, a State cooperating inspector can witness and document the disposal.

Once it becomes clear that a property is infected with a citrus greening pathogen, known infected trees are to be removed to eliminate them as inoculum reservoirs. After assessment and an effective delimiting survey, control or containment of the disease may include removal and destruction of exposed plants within buffers. The buffer distance is at the time of this publication difficult to determine because of latency of the disease, lack of sensitive enough diagnostic tools, and unknown dispersal distance for the psyllid vectors. Recommendations from the HLB Science Panel, may include removal of entire citrus blocks in groves with known infections and general psyllid movement within an area.

If there is a confirmed detection in an area with psyllid populations, control strategies will be determined by federal and state regulatory officials in consultation with a scientist advisory panel consisting of members knowledgeable of the disease and its epidemiology. Likewise, insect vector control decisions will also be made based on the type of property infested in consultation with the science advisory panel.

Control of Huanglongbing Disease

Known infected trees are to be removed and destroyed as no “cure” is known for greening. While treatment with antibiotics has been shown to suppress symptoms, it is impractical, can be phytotoxic, and does not eliminate the bacteria from the tree.

Various methods have been attempted in other countries to control infestations of HLB in citrus, however the disease has never been successfully eradicated or suppressed in the short-term. While long-term management techniques are available and are being further tested, new detections require quick action, and the following guidelines can minimize the spread and reduce the incidence of infection in an area if caught early enough.

Citrus growing states or areas where no known citrus psyllid vectors present:

Because HLB is a self-limiting bacterial disease spread only by the vector or grafting, it is likely that a positive detection in an area where the psyllid vector is not known to occur will represent an isolated infection. The infected plant may have been illegally imported from a country or state with HLB and planted there, or grafted from such a plant. It is important to trace all budwood or other grafting sources associated with positive detections from commercial groves or nurseries, and conduct thorough traceback-traceforward investigations for positive trees from residential areas.

Any plant of the same species in the area should be sampled and tested in case it was grafted from the same source of infected material. Remove and destroy all known infected plants and suspect associated host plants that may have come from the same budwood source but not showing symptoms.

Place under quarantine nurseries, other entities with sources of budwood, or trees that were traced from positive detections, and do not allow shipping of host material from those properties or from a quarantined area. Suspect properties with associated grafting from infected sources can be quarantined until a thorough investigation determines the level of risk for those properties shipping propagative material out of the area.

Citrus growing states or areas with known citrus psyllid infestations:

In citrus growing areas where the vector is present, effective early detection and eradication is likely not achievable because 1) infections can be latent in a plant with symptoms appearing six months to two years after infection, and 2) current diagnostic tools for detecting latent infections are not sensitive enough to allow confident delineation of the infection in an area.

If the overall severity of the disease in a geographic area can be assessed and determined to be low or isolated, removal of infected trees will eliminate sources of inoculum. Removal of exposed trees with in a buffer area is not practical at this time for reasons stated above. If effective tools

were available and validated, an aggressive eradication program would involve removing exposed trees around positive testing trees in a large buffer area. The size depends on epidemiology of the disease and vector's dispersal ability. The dispersal of the African citrus psyllid, *Trioza erytreae* is thought to be approximately 1.5 kilometers, however only anecdotal information on the Asian citrus psyllid, *Diaphorina citri*, is known. Dispersal behavior may be density dependent. Long distance dispersal is known to occur with psyllids picking up wind currents.

Control of Psyllid Vectors

Vector control can include both chemical and biological control methods. Several insecticides, such as imidacloprid or fenpropathrin (See Appendix 6) have been shown to provide effective control of the psyllid vectors. The methods and insecticide dosages for larger trees for control of vectors in mature groves should be reviewed with the science panel prior to implementation.

Psyllid populations are monitored, either by scouting or by yellow sticky boards, and citrus trees are sprayed with proper timing to control the psyllids. Be aware of population trends of psyllids in infested areas before treatments. Populations build up during flushing periods and may also be influenced by populations on nearby ornamental hosts. Densities may also be higher at the edges of groves.

Vectors should be controlled in advance of any destruction actions to minimize spread when destruction of infected trees occurs. This prevents dispersal of infected adults during tree cutting operations.

Before conducting any insecticide treatments, assure the proper environmental documentation is in place and environmental monitoring has been arranged if appropriate. Coordinated outreach and notifications of quarantine actions will minimize opposition by landowners and residents. These efforts are to be closely coordinated with State agencies responsible for pesticide applications and licensing.

Control Procedures for Positive Nursery Detections

The following guidelines may be used in nurseries in the event the first detection in an area occurs there and there is no evidence of a general infection in surrounding areas.

Several actions need to occur immediately upon confirmation that a citrus nursery sample is positive for citrus greening: 1) If not done at the time of sampling, an immediate quarantine hold must be placed on the nursery to prevent the movement of trees and budwood from the premises. 2) Check nursery records to obtain names and addresses for all sales during the prior six months. These should be grouped in three categories a) sales in the past month, b) sales in the two months prior to that, and c) sales in the three months prior to that, for a total of six months. 3) Evaluate the disease

situation, including identification and inspection of the budwood source(s) of the diseased tree(s). 4) Commence vector control and tree removal operations as soon as possible.

When evaluating the disease situation, consider the following factors.

1) What is the source of the infected trees?
2) Have plants from the same sources been distributed or sold to other areas.
3) Within the nursery, how many and what is the distribution of trees showing symptoms?
4) Are the diseased trees (assuming more than one) clustered together or randomly scattered? If clustered, does it suggest possible use of infected budwood or (more likely) tree-to-tree movement of the vectors?
5) Were any symptoms present on the budwood source tree(s)?
6) Are vectors present and, if so, in what numbers?
7) Has the nursery maintained a good vector control program?
8) Are alternative (non-citrus) hosts of the vector (e.g., orange jasmine in the case of Asian citrus psyllid) present on or near the property?
9) Are there any citrus trees on the properties immediately adjacent to the nursery and, if so, do any of the trees show symptoms of HLB?
10) Do the trees in the nursery appear to be healthy and growing vigorously?
11) What grafting practices have taken place? What are the sanitary measures taken by propagators in the nursery?

In consultation with the HLB Science Panel, consideration should be given by state and federal regulatory officials to removing all trees, depending on the size and physical layout of the nursery. If it is a large nursery with trees set out in blocks, with some separation between blocks, and the infected trees are all within one or two blocks, remove all trees within the blocks plus any trees in adjacent blocks of with infected trees. If vectors are known to occur in the area, all trees in the nursery should be treated with an approved insecticide prior to commencement of removal operations.

Following completion of the tree removal operations, the trees in the nursery should be inspected on a bi-weekly schedule for the next two months. If no additional positive trees are found, inspect on a monthly schedule for the remainder of the quarantine period. If the trees appeared healthy and vigorous, a six-month hold after the last positive is removed should be adequate for infected trees to show symptoms. Any infected trees found during subsequent inspections will reset the clock on the hold period. The nursery should maintain a good vector control program during this period, including removal of alternative hosts of the vector present on the nursery property.

If the psyllid vector occurs in the area, citrus nurseries may be required to locate operations to greenhouses to protect from infective vectors.

If certain groups of trees would be beyond prime condition for sale at the end of the hold period, especially if it goes beyond six months, the nursery may voluntarily elect to destroy those trees to avoid further expenses involved with their maintenance. Small nursery operations may voluntarily choose to destroy all trees for economic reasons, rather than maintain them for the six-month (or longer) hold period.

Tracebacks of prior sales of nursery stock should begin as soon as possible, with initial survey directed at trees sold in the previous month. When completed, go to the second group, sales in the two months before that. If no greening-infected trees are found in either group, it may not be necessary to inspect the third group. If, however, positive trees are found, the third group should also be inspected.

Tree Removal and Disposal

Standard tree removal methods can be employed, making certain that the trees have be sprayed with an insecticide prior to removal to kill any psyllid life stages which may be present. Physical removal of the trees can occur by pulling, pushing the tree out of the ground with heavy equipment. If this technique is used, plants may later sprout from roots left in the ground, and these must be controlled with an approved herbicide (glyphosate). Trees can also be removed by cutting them at or near the soil line. If the latter method is employed, the freshly-cut stump should be treated with an herbicide to kill it and minimize root sprouting (Tordon®, Garlon®). Check the labeling to see that this use meets requirements.

Unlike many other citrus pathogens, greening bacteria are spread only by grafting with infected budwood and by the two psyllid vectors. Therefore, any method of disposal which kills any vectors present, prevents further access to foliage by vectors, and prevents usage of removed trees as budwood sources is appropriate. Suitable disposal methods include burning, chipping (only smaller diameter branches and foliage would need to be chipped; large diameter wood could be disposed of by other means), or burial in a landfill.

Control of Hosts of Insect Vectors

Another major component of an effective control program is the removal of preferred alternative hosts of the vectors. In the case of *D. citri* in Florida and Texas, this would involve the removal, for example, of any orange jasmine *(Murraya paniculata)* plants growing near citrus plantings, and especially any growing near citrus nurseries.

Nurseries that wish to ship any insect vector hosts from a regulated property to citrus growing states must do so under a compliance agreement that requires prescribed treatments to eliminate the risk of spreading the insect vectors. (See the Regulatory section)

Biological Control of Psyllid Vectors

Biological control has been reported effective in several countries, using a variety of parasites and predators. Biological control of the two psyllid vectors of citrus greening was achieved on Reunion Island (France: Indian Ocean) with hymenopteran psyllid parasites: *Tamarixia radiate*, introduced from India against *Diaphorina citri*, and *Tamarixia dryi*, from South Africa, against *Trioza erytreae*. *T. radiata* is reported to attack the Asian citrus psyllid reaching a level of 95% parasitization in North India, 90% in South India, and with the addition of *D. aligarhensis* reaching 92.5% in Reunion Island. It has been reported reaching nearly 100% parasitization in Taiwan.

The two parasites specific to *D. citri* were introduced into Florida in 1999, but only one, *T. radiata*, is established. Also in Florida, populations of the native ladybeetle *Olla v-nigrum* have increased significantly since the introduction of the Asian citrus psyllid, and this ladybeetle, together with the Asian multicolored ladybeetle *Harmonia axyridis*, have been found preying on *D. citri* throughout its range in Florida. Fungi also attack *D. citri* in Florida's warm, moist climate. Biological control, however, may not be sufficient to adequately reduce insect populations, especially during the early spring months or in nurseries where trees are constantly putting on new growth.

The use of biological control agents can reduce citrus psyllid population, density pressure, and subsequently the rate of distribution of the psyllid throughout the citrus growing region. Lower psyllid populations may subsequently reduce the incidence of the disease the spread of the disease, however its affect on disease incidence is unknown. In an integrated pest management (IPM) program within a commercial citrus grove, biological control can be one component of a control strategy. Biological control alone in residential areas with citrus or ornamental psyllid hosts may provide some control of the vector that may reduce psyllid migration into adjacent areas such as commercial groves. This type of a classical biological control program can provide a self-sustaining control technology against this psyllid pest. Commercial citrus groves may find the impact of the parasitoids less effective when indiscriminate pesticides are used that may kill the parasitioids, other natural enemies, and the psyllid. Many effective insecticides will not be compatible with natural enemies and must be carefully selected and applied in order to maximize all control components of the IPM program including cultural practices and removing diseased trees.

Long-term HLB Management

If eradication or containment is not feasible, a management program such as that in South Africa, which utilizes a multi-pronged approach, may allow production to continue. In this integrated approach, 1) alternative hosts of the vector are removed throughout the production area to minimize vector population carryover when the citrus is not flushing, 2) new plantings are

made with healthy trees propagated from tested budwood source trees, 3) groves are monitored to detect any vector population buildup, generally by scouting or use of yellow sticky boards, and 4) detection triggers prompt chemical treatment to control vectors. Finally, 5) groves are regularly inspected to detect greening symptoms as early as possible and infected trees are promptly removed.

Control Records

Also attach any documentation, receipts, etc. that document these actions. Program personnel must maintain records and maps noting the locations of all detections, the number and type plants subjected to control actions, and the materials and formulations used in each treated area. Attach all documentation to the office EAN copy.

Environmental Monitoring

Contact PPQ headquarters for guidance on environmental documentation and monitoring.

Overview

A key element in designing a program or an emergency response is consultation with Environmental Services (ES), a unit of APHIS' Policy and Program Development Staff (PPD). ES prepares environmental documentation such as environmental impact statements (EIS) and environmental assessments (EA) to aid in program operational decisions, as well as Endangered Species consultation ES also coordinates pesticide registration and approvals for APHIS pest control and eradication programs, ensuring that registrations and approvals meet program use needs and conform to pesticide use requirements. Refer to the Resources Section of this document for additional information.

Disclaimer

All uses of pesticides must be registered or approved by appropriate Federal, State, and/or Tribal agencies before they can be applied. The information provided on pesticide labels may not reflect all of the actual information, including precautions and instructions for use, which you are required to follow in your specific State or locality. It is the responsibility of persons intending to use a pesticide to read and abide by the label, including labeling that has been approved for the particular State or locality in which the chemical is to be used, and to comply with all Federal, State, Tribal, and local laws and regulations relating to the use of the pesticide. APHIS program staffs are responsible for their compliance with applicable environmental regulations.

National Environmental Policy Act

Agencies should prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) concurrently and integrated with environmental impact analyses, surveys, and studies required by the Fish and Wildlife Coordination Act, National Historic Preservation Act of 1966, Endangered Species Act, and other laws and executive orders. Environmental document prepared to comply with other acts also may be incorporated into National Environmental Policy Act (NEPA) documents as part of the NEPA process.

Categorical Exclusions

Categorical exclusions (CE) are categories of actions that do not have a significant effect on the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is generally required.
APHIS managers are encouraged to use categorical exclusions where appropriate to reduce paperwork and speed the decision making process. Proposed actions are subject to sufficient environmental review to determine whether they fall within the broadly defined categories. Each time a specific categorical exclusion is used, the required review must be done. An EA may be prepared for proposed

actions otherwise excluded when the manager determines that the action may have potential to significantly affect the environment or an EA would be helpful in planning or decision making.

Environmental Impact Statements

An environmental impact statement (EIS) is a detailed statement that must be included in every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment. The primary purpose of an EIS is to serve as an action-forcing device to insure that the policies and goals defined in the National Environmental Policy Act (NEPA) are infused into the ongoing programs and actions of the Federal government. Generally, EIS's are prepared when Federal agencies recognize that their actions have the potential for significant environmental effects (adverse or beneficial), or when an environmental assessment leads to a finding of potential significant impact .

APHIS prepares EIS's for administrative proceedings that establish broad scale significant impact-generating strategies, methods, or techniques such as large-scale aerial pesticide applications. This can include contingency or emergency strategies that are comprehensive in scope or long-range plans with potential for significant environmental impact. APHIS also prepares programmatic EIS's to examine strategies and options for dealing with issues with important implications for the maintenance and enhancement of environmental quality.

Environmental Assessments

An environmental assessment (EA) is a concise public document that briefly provides sufficient evidence and analysis for determining whether to prepare an environmental impact statement (EIS) or finding of no significant impact (FONSI). An EA aids an agency's compliance with the National Environmental Policy Act (NEPA) when no EIS is necessary and facilitates the preparation of an EIS when necessary. Generally, an EA leads to a FONSI or an EIS, but it could also lead to abandonment of a proposed action.

The content of an EA must include brief discussions of the need, alternatives, and potential environmental impacts of the proposal a list of agencies and persons consulted.

Environmental Monitoring

PPQ requests assistance from ES before PPQ personnel or funding are used for control operations. Additionally, program staff should consult with the PPQ PDMP ISPM Environmental Monitoring staff to determine if an environmental monitoring plan is required for the operation. State, regional, and national program managers determine counties where treatments may be needed.

Program personnel should evaluate the success of biological control agents and herbicide treatments used in eradication or suppression of the target FNW or host weeds and avoid damage to non-target plants.

Biological Assessment

A biological assessment (BA) is an analysis of the effects that a Federal agency action may have on listed or proposed endangered or threatened species and designated critical habitat. The Endangered Species Act (ESA) requires this analysis if the proposed action may affect a listed species. In such a case consultation with the U.S. Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS) is required. Federal agencies are required to insure that any action authorized, funded, or carried out is not likely to jeopardize listed species or result in adverse modification of designated critical habitat.

DEFINITIONS

Blotchy mottling	The most characteristic symptom of huanglongbing disesase on citrus leaves caused by infection by *Candidatus* Liberibacter species. These symptoms appear on both sides of a leaf as varying chlorotic patches that may pass through the leaf veins.
Chlorosis	Yellowing of normally green tissue due to chlorophyll destruction in infected plants.
Decontamination	The application of an approved chemical or other treatment to contaminated implements, material, or buildings for killing or deactivating a pathogen.
Delimiting Survey	After the initial first detection in a area, this type of survey is conducted to define the geographic range of the infection/infestation.
General Detection Survey	A survey conducted over a large area to discover new potential infestations/infections in areas where the pest/disease is not known to occur.
Host	A plant which is invaded by a parasite or pathogen and from which it obtains its nutrients.
Identification Authority	Authority to confirm the presence of a particular pest organism issued by the APHIS National Identification Services to diagnosticians that have demonstrated proficiency in identifying.
Infection	The establishment of a parasite on or within a host plant.
Monitoring or Evaluation Survey	A survey conducted at a site where a disease was found and where an eradication program is being performed.
Necrosis	Dead or discolored plant tissue.
Pathogen	Any organism that can incite a disease.
PCR	An acronym for Polymerase Chain Reaction, and laboratory technique that amplifies DNA sequences in order to determine if a host is infected with a known pathogen.
Phloem-limited	The quality of a pathogen that describes its ability to only survive within the phloem vascular system of a plant.

Potentially Actionable Suspect Sample	Also know as PASS, a presumptive positive sample diagnosed or identified by provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation.
Presumptive Positive	Such a result may require confirmatory testing if the sample is a PASS sample.
Rapid Delimiting Survey	A survey method after a first detection in an area, deploy quickly with teams in designated increments (in radii or linear) away from the known focus detection.
Rutaceous	In the plant family, Rutaceae.
Sentinel Survey	A survey method that designates particular plants, of susceptible species or varieties, for repeated visits over a predetermined time period.
Symptom	The external and internal reactions or alterations of a plant as the result of a disease.
Targeted Survey	Choosing an area, usually residential, to concentrate surveys based on known pathway information from source countries with zip code based demographic information, also known as a “hot zone” survey.
Traceback	To investigate the origin of infestated plants through intermediate steps in commercial distribution channels to the origin.
Traceforward	To investigate where infected plants may have been distributed from a source through steps in commercial distribution channels.
Vector	Carrier of an infectious agent; capable of transmitting infection from one host to another; especially the animal that transfers an infectious agent from one host to another, usually an arthropod.

Appendix 1 Psyllid Vectors, Feeding Damage, and HLB Symptomology on Hosts

Insect Vectors of Huanglongbing (HLB)

Figure 1. Asian citrus psyllid, *Diaphorina citri* Kuwayama, in typical feeding position with raised abdomen. Photo courtesy of David Hall, USDA-ARS, Ft. Pierce, FL

Figure 2. African citrus psyllid *Trioza erytreae* (Del Guercio) with eggs. Photo courtesy of S. P. Van Vuuren, Citrus Research International, South Africa

Figure 3. Asian citrus psyllid, *Diaphorina citri* Kuwayama, nymphal instars. Photo courtesy of David Hall, USDA-ARS, Ft. Pierce, FL

Figure 4. African citrus psyllid *Trioza erytreae* Del Guercio nymph. Photo courtesy of Peter Stephen, Citrus Research International, South Africa

Foliar Symptoms of Feeding by African Pysllid

Figure 5. Citrus leaves, top view, showing pit galls produced by African citrus psyllid, *Trioza erytreae* Del Guercio. *FAO website*

Figure 6 . Citrus leaves, underside, showing pit galls produced by African citrus psyllid, *Trioza erytreae* Del Guercio. *FAO website*

Figure 7. Citrus leaves showing pit galls produced by African citrus psyllid, *Trioza erytreae* Del Guercio. Photo courtesy of Peter Stephen, Citrus Research International, South Africa

Figure 8. Close up of citrus leaves showing pit galls produced by African citrus psyllid, *Trioza erytreae* Del Guercio. *FAO website*

Tree and Foliar Symptoms of HLB on Citrus

Figure 9. Presence of yellow shoots typical of Huanlongbing. Photo courtesy T.R. Gottwald and S.M. Garnsey.

Foliar Symptoms of HLB on Citrus

Figure 10. Leaf mottling caused by Haunglongbing. Photo courtesy J.M. Bové and M. Garnier. Reprinted from Timmer, L.W., Garnsey, S.M., and Graham, J.H. 2000, Compendium of Citrus Diseases, Second Edition, American Phytopathological Society, St. Paul, MN.

Figure 11. Close-up of one leaf showing vein yellowing and mottle. (From: http://www.doacs.state.fl.us/pi/enpp/germplasm/exotics.html)

Figure 12. Vein yellowing in HLB-infected leaf (top); normal leaf on bottom.

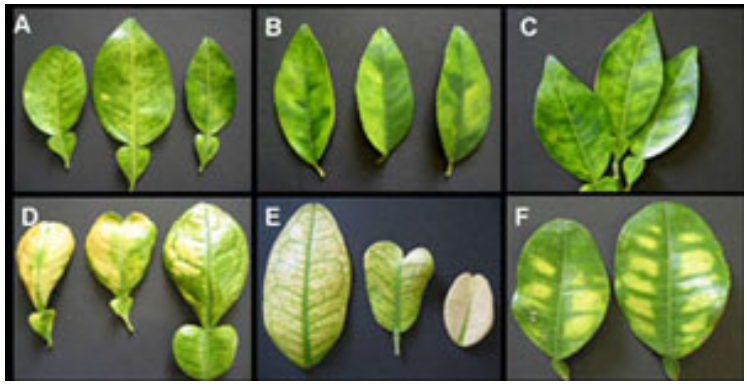


Figure 13. Symptoms of Citrus Hunglongbing (greening) in Florida . The symptoms are characterized with yellow mottles, and vary from different host plants. A: Sour orange (*Citrus aurantium)* ; B: Lime (*Citrus aurantifolia)* ; D: unknown (Citrus sp.); C, E, and F: Pummelo (*C. maxima)* Image by Xiaoan Sun, Florida DOACS, Division of Plant Industry.

Other Foliar Symptoms That May be Confused for HLB

Figure 12. Zinc deficiency symptoms in citrus.

Symptoms of HLB on Fruit

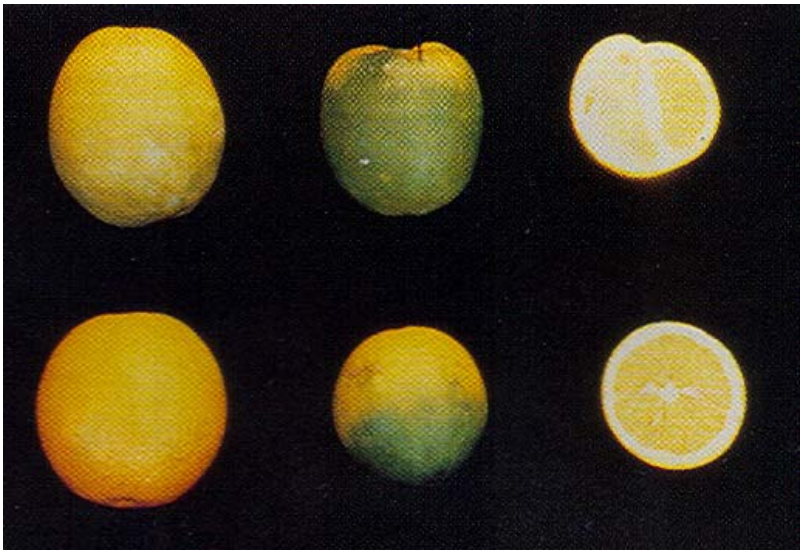


Figure 13. Misshapen and greenish fruit of citrus affected with HLB compared to health fruit shown in the lower left. Photo courtesy P. Broadbent. Reprinted from Timmer, L.W., Garnsey, S.M., and Graham, J.H. 2000, Compendium of Citrus Diseases, Second Edition, American Phytopathological Society, St. Paul, MN.

Figure 14. Symptoms of HLB on mandarin orange fruit. Photo courtesy T.R. Gottwald and S.M. Garnsey.

Figure 15. Cross section of HLB symptomatic fruit. Photo courtesy of Xiaoan Sun, Florida Department of Agriculture and Consumer Services

Appendix 2

Psyllid Identification **Huanglongbing, Citrus Greening Disease**

Collection and Preparation of Specimens

The vectors of citrus greening pathogens are indicators of areas at risk for the disease. The psyllid vector may occur in the area but the vector may not be carrying the pathogen. Psyllid identification is important as a possible indicator of disease presence in a geographical area. Currently methods exist to detect the presence of the pathogen within the vector itself. Collecting and analyzing vectors from an area can be an important method for learning about citrus greening epidemiology.

Collect as many specimens, adults and nymphs, as possible for identification, by the local designated identifier and/or subsequent PCR analysis of the vector for presence of the pathogen. Do not mix samples. Be sure to separate insects into vials by tree or location. Prepare the PPQ form 391 (Specimens for Determination) and be sure to include information as noted:

- date of collection;
- sample number from predetermined numbering system;
- collector's name and agency;
- full address including county;
- type of property, i.e., residential, nursery, commercial grove, feral or abandoned grove;
- grower's field ID numbers, if appropriate;
- GPS coordinates of the host plant and property;
- host species, and cultivar;
- observations of the number of trees showing symptoms;
- general conditions or any other relevant information.

Prepare specimens according to the following protocols:

- gather nymphs/adults from the host plant, place in the same vial;
- label the vial with a sample number, date, locale, etc.;
- preserve the insects in 70 % ethyl alcohol (95% for PCR analysis);
- Fed-Ex vials in well-padded box, with absorbent materials in case of vial breakage or leaks, and place in a ziplock bag;
- include a completed PPQ form 523 (with the submitter's e-mail address on the form).

Submit specimens to your state or cooperating university entomologist for screening. When the suspect specimen represents a potentially new detection for a state, please forward to the appropriate specialist for confirmation (Table 3.1). Include PPQ Form 391 (Specimens for Determination) marked "Urgent" (see PPQ General Operational Procedures Manual M390.500) with all specimens.

Appendix 2

Psyllid Identification **Huanglongbing, Citrus Greening Disease**

Table 3.1 , Information Flow for the Identification of Specimens	
Identification	State PPQ Laboratory Area Identifier
Confirmation	Leader, Taxonomic Services Unit, USDA, ARS, BA, PSI Building 005, Room 137, BARC-West 10300 Baltimore Avenue Beltsville, MD 20705-2350
Notification	Confirmation Notification to Requesting Party and Other USDA Agencies—State and Territory Agricultural Regulatory Officials

Asian Citrus Psyllid, *Diaphorina citri*

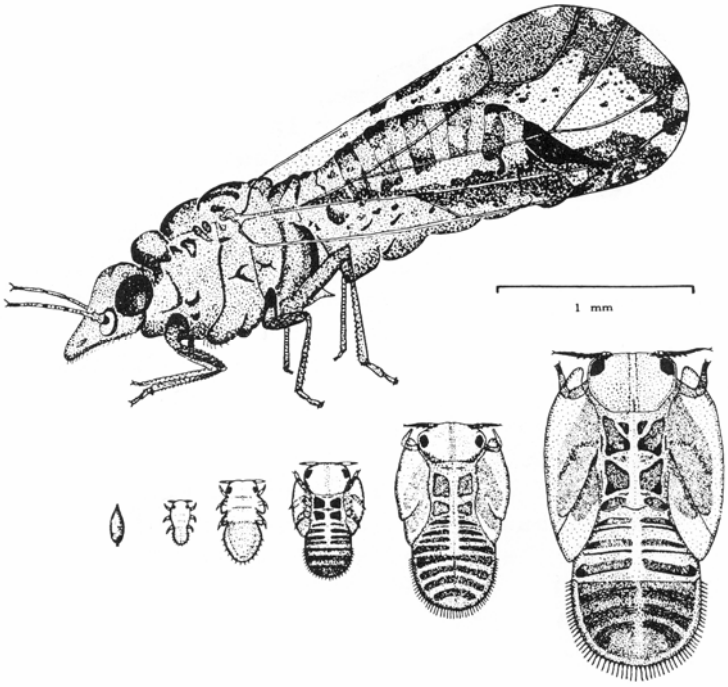


Figure ___. Adult and nymphal instars of the Asian citrus psyllid, *Diaphorina citri* Kuwayama. Illustration from Catling, 1970.

Adults of the Asian citrus psyllid, *Diaphorina* citri Kawayama 1908 (=*Eupharalus citri*) can be separated from most other *Diaphorina* species on citrus and citrus relatives by distinctive wing patterning. Halbert and Manjunath 2004 give a review of the biology and taxonomic references for these other species. They also list several other psyllid genera typically found on citrus.

Appendix 2

Psyllid Identification **Huanglongbing, Citrus Greening Disease**

African citrus psyllid, *Trioza erytrea*

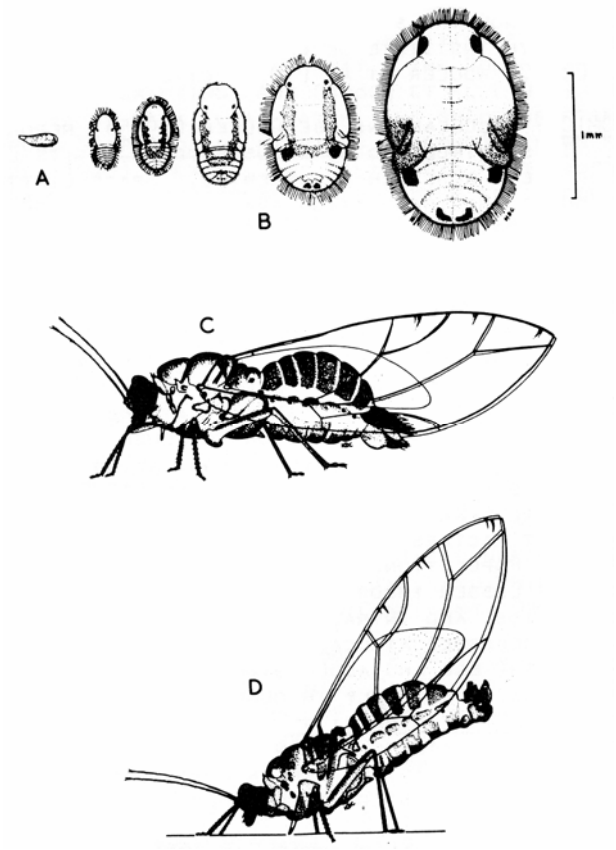


Figure ___. Egg (A), nymphal instars (B) and adults (C and D) of the African citrus psyllid, *Trioza erytreae* (del Guercio). Illustration from Catling and Annecke, 1968.

The African citrus psyllid, *Trioza erytreae* (del Guercio) 1918 is difficult to separate morphologically from ten other species in the genus, but can be separated by host preferences (Halbert and Manjunath, 2004).

Disinfection of Pruning Equipment used in Sampling

Overview

Pruning shears used to cut samples should be disinfected prior to use on a new property (and preferably before use on each tree) to avoid spreading citrus exocortis or other citrus viroids. These citrus pathogens can be carried on the cutting surfaces of pruning shears, knives, and other implements used for cutting and pruning operations. Making a cut on an infected tree is sufficient to contaminate the cutting tool; subsequent cuts on other trees will introduce the viroid and infect the tree. Viroids, small pieces of "naked" RNA similar to a plant virus but lacking the protein coat, are extremely difficult to remove from the tool and are not "killed" (inactivated) by most disinfectants or even by high heat.

A spray, or brief immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (common household liquid bleach) is an effective way to inactivate citrus viroids and prevent their spread.

CAUTION: Household liquid bleach is very corrosive. Avoid spilling or splashing it onto skin, eyes, or clothing. Note all precautions on the bleach container label.

Usage Instructions

Use a fresh bottle of household bleach, since sodium hypochlorite solutions "break down" over time. The bleach label should indicate the concentration of sodium hypochlorite in the bleach. Use a brand of liquid bleach which contains a 5% sodium hypochlorite solution as it comes from the factory (this is a common strength). If stronger, the bleach will need to be diluted with water to achieve a final working concentration of 5% sodium hypochlorite.

Use a thick-walled non-breakable plastic container for the bleach, with a top opening large enough to easily dip the cutting surfaces of the pruning shears into the bleach. Alternatively, the bleach can be kept in a spray bottle. If a dipping method is used, pour sufficient bleach into the container to allow easy dipping of the cutting surfaces of the pruning shears. The bleach should be replaced every 2-3 hours, as it "breaks down" when exposed to the air.

When sampling is completed for the day, disinfect the shears by dipping in the bleach, then rinse thoroughly with water to remove all bleach solution. To minimize the corrosive effects of the bleach on the pruning tool, dry it after the water rinse and coat the cutting surfaces with a thin film of lubricating oil.

PPQform391

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions. *See reverse for additional OMB information.*

FORM APPROVED OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER

2. DATE (MO / DA / YR)

3. SUBMITTING AGENCY
☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER

6. ADDRESS OF SENDER

ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*

7. NAME AND ADDRESS OF PROPERTY OR OWNER

COUNTRY/ COUNTY

PURPOSE

8. REASON FOR IDENTIFICATION *("X" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS*)*
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
F. ☐ Possible Immigrant *(Explain in* REMARKS*)*
G. ☐ Survey *(Explain in* REMARKS*)*
H. ☐ Other *(Explain in* REMARKS*)*

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

HOST DATA

10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure and indicate* ☐ Number ☐ Percent*):*

12. PLANT DISTRIBUTION
☐ LIMITED ☐ SCATTERED ☐ WIDESPREAD

13. PLANT PARTS AFFECTED
☐ Leaves, Upper Surface ☐ Leaves, Lower Surface ☐ Petiole ☐ Stem
☐ Trunk/Bark ☐ Branches ☐ Growing Tips ☐ Roots
☐ Bulbs, Tubers, Corms ☐ Buds ☐ Flowers ☐ Fruits or Nuts ☐ Seeds

PEST DATA

14. PEST DISTRIBUTION
☐ FEW ☐ COMMON ☐ ABUNDANT ☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

SIGNATURE

DATE

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

PPQ FORM 391 *Previous editions are obsolete.*
(AUG 02)

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

PPQ form 523

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED - OMB NO. 0579-0102

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION | 2. DATE ISSUED

3. NAME AND QUANTITY OF ARTICLE(S)

4. LOCATION OF ARTICLES

5. DESTINATION OF ARTICLES

6. SHIPPER

7. NAME OF CARRIER

8. SHIPMENT ID NO.(S)

9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. | FAX NO.

SS NO. | TAX ID NO.

10. PORT OF LADING | 11. DATE OF ARRIVAL

12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)

12a. PEST ID NO. | 12b. DATE INTERCEPTED

13. COUNTRY OF ORIGIN | 14. GROWER NO.

15. FOREIGN CERTIFICATE NO.

15a. PLACE ISSUED | 15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT: _____

☐ RE-EXPORTATION: _____

☐ DESTRUCTION: _____

☐ OTHER: _____

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):

18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE: | DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER: | DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

For more information on this section, please contact the state plant health director of USDA, APHIS PPQ in your state or the plant health section of your State Department of Agriculture.

Registered Insecticides for Control of Citrus Psyllids

There may be other products registered for use in various situations for citrus psyllids, Check with APHIS Environmental Services for information on other insecticide labeling.

Trade Name	**% Active Ingredients**	**EPA Reg. No.**	**Use Sites**
Marathon II	Imidacloprid, 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine (21.4%)	3125-549-59807	Ornamentals, Fruit and Nut Trees, and Vegetable Plants in Greenhouses, Nurseries, and Interior Plantscapes
Marathon 60 WP	Imidacloprid, 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine (60%)	3125-492-59087	Ornamentals and Vegetable Plants in Greenhouses, Nurseries, and Interior Plantscapes
Tame 2.4 EC	Fenpropathrin (30.9%)	59639-77	Commercial Use on Indoor and Outdoor Ornamental and Nursery Plantings
Dursban 4E	Chlorpyrifos [0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] (44.8%)	655-499	Fruit, Nut and Citrus Trees, Golf Course Turf and Commerical Nursery Plants
Discus	Cyfluthrin (0.70%) Imidacloprid (2.94%)	432-1392-59807	Ornamentals, Non-Bearing Fruit and Nut Trees, In Field and Container Nurseries
Chlorpyrifos G-Pro 4	Chlorpyrifos [0, 0-dimethyl 0-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] (44.7%)	79676-9	Commercial Nurseries and Greenhouses; Golf Course Turf, Turf and Ornamentals Around Industrial Buildings; Turf and Ornamentals in Road Medians

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